Vol. XIII, No. 4 November, 1926

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF ECONOMIC BIOLOGISTS

BY

W. B. BRIERLEY

AND

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CAMBRIDGE UNIVERSITY PRESS
LONDON: FETTER LANE, E.C. 4
also
H. K. LEWIS & CO., LTD., 136, GOWER STREET, LONDON, W.C. 1
WHELDON & WESLEY, LTD., 2, 3, 4, ARTHUR ST., NEW OXFORD ST., W.C. 2
PARIS: LIBRAIRIE HACHETTE & CIE.
CHICAGO: THE UNIVERSITY OF CHICAGO PRESS
(AGENTS FOR THE UNITED STATES)
BOMBAY, CALCUTTA, MADRAS: MACMILLAN & CO., LTD.
TOKYO: THE MARUZEN-KABUSHIKI-KAISHA

Price Twelve Shillings net

PRINTED IN GREAT BRITAIN

The Association of Economic Biologists

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VOLUME XIII NOVEMBER, 1926 No. 4

INVESTIGATIONS INTO INSECTICIDES FOR ROOT MEALY BUG AND ROOT APHIS

BY WINIFRED H. SAUNDERS.

(*Wolfe-Barry Research Scholar in Entomology at the Imperial College of Science*, 1915; *Lecturer in Biology at Goldsmiths' College, University of London.*)

WITH AN INTRODUCTION
BY THE LATE PROFESSOR H. MAXWELL-LEFROY.

INTRODUCTION.

THE following paper summarises the results of work done by Miss W. H. Saunders and myself to find a convenient safe treatment for pot plants in greenhouses against the root mealy bug and root aphis.

The work was begun in 1915, at the Royal Horticultural Society's Gardens at Wisley, at the Chelsea Physic Garden and at the Imperial College of Science, South Kensington.

Miss Saunders was enabled to work in 1915 on the scholarship generously given by Sir John Wolfe-Barry: the work was not completed when I went to India at the end of 1915 and Miss Saunders resumed in 1917 in her vacation.

So far as the present conclusions are concerned they have been arrived at by subsequent work at the Chelsea Physic Garden during 1917. Between the cessation of work in 1915 and its resumption in July, 1917, the detailed examination of a very long series of chemical substances in relation to their killing power on maggots had been made and from these I selected twenty new substances, not known to me in 1915, for test during 1917.

We have not, therefore, exhaustively tested all the substances available but we have selected likely ones, aiming at rapidly securing some practical treatment which could be applied at once at the Chelsea Physic Garden.

This enquiry is linked up with several others, all directed to determining the insecticidal action of a variety of substances, with a view to securing information as to how far insecticidal action can be correlated with chemical composition.

The enquiry into the insecticidal action of substances on maggots is one, those relating to killing house flies, white fly (*Aleurodes*) and mealworms are others. The fact that these results are part of a connected whole explains their apparent scrappiness and incompleteness.

We wish to acknowledge our indebtedness to Sir John Wolfe-Barry, whose generosity made possible this investigation: and also to acknowledge the assistance received from Mr Hales, Curator of the Physic Garden, Chelsea, and the Royal Horticultural Society for the facilities given us at Wisley.

H. M. Lefroy.

Note. The introduction to this paper by the late Professor Maxwell-Lefroy explains the part he has played in its production. The paper, almost in its present form, was completed before his death and the changes I have since made have been in the inclusion of some more of his results and in certain slight modifications suggested by Professor Balfour-Browne, who kindly looked through the manuscript.

THE ROOT MEALY BUG AND ROOT APHIS.

The investigation with which this paper deals was suggested by the extent of the damage caused to pot plants by root-feeding insects and by the difficulties experienced by growers in selecting a satisfactory remedy.

An attempt has been made to solve the problems arising from conditions under which greenhouse pot plants are grown, in connection with combating certain root pests which are parasitic on them.

This paper deals with two types of insect both of which are common pests on the roots of plants.

1. Mealy Bug (2 species)[1].
2. Lettuce Root Aphis—*Pemphigus lactucarius* (Pass.) [= *P. bursarius* (L.)].

The particular problem to be solved was the discovery of a remedy which would act favourably upon the plant and destroy the pest and it was first necessary, therefore, to gain some knowledge of the life-history and habits of the insects and of the resistance of the plant hosts to certain chemicals. To trace the complete life-histories of the insects

[1] These were originally identified as *Ripersia halophila* and *R. terrestris* but identical material of the first species has since been examined by Mr E. E. Green, who describes it as a new species in a paper entilted "Observations on British Coccidae," which will appear shortly in *The Entomologist's Monthly Magazine.*

was beyond the scope of this undertaking. The mealy bug material used for the experiments was obtained from the Chelsea Physic Garden, the second species of mealy bug was sent from a private garden, whilst the aphis was supplied from the Royal Horticultural Society's Gardens at Wisley.

To ascertain whether the infected hosts at the Chelsea Physic Garden had a pest in common or whether additional species were present, specimens were collected from every species of infected plant. Twenty-nine species of pot plants in one greenhouse were found to be infected with *Ripersia* sp. and of these the leguminous species, especially *Acacias*, were the favourites. The *Ripersia* species are typical mealy bugs. The species just referred to is a serious greenhouse pest, using many genera and species of plants as hosts. This parasite is visible at all seasons of the year attached to the roots of plants. The insect applies itself firmly, clinging with the aid of its claws and absorbing nourishment by means of its sucking mouth parts, which penetrate the tissues of the plant. The insects are produced in large numbers and females and nymphs actively wander over the roots. The females are viviparous and the nymphs leave the ovisacs and become independent soon after birth.

By frequenting the outer portion of the soil, the insects come into contact with the youngest roots, thus inflicting serious damage. In all stages the bugs travel from pot to pot. They were seen on their migrations between some pots which were placed on brown paper. Owing to this restless habit, clean plants become infected when they are placed amongst infected ones standing on such materials as gravel and cinders, which provide shelter for the bugs.

Pemphigus lactucarius, though differing from *Ripersia* in structure and habits, adopts a similar mode of attack on its particular plant hosts. It is a common pest on lettuce, though the material used in this investigation was found attacking *Primula* species in pots and in rockeries and pot plants of *Chrysanthemum leucanthemum*.

The insects cause considerable damage to valuable alpine species of *Primula*; they attack the younger roots more particularly.

The aphids found on these plants were wingless viviparous females—neither winged females nor males were found.

Nymphs. Mature specimens of *Pemphigus lactucarius* have five antennal segments, while the nymphs have four.

TREATMENTS.

Various species of *Acacia* (the favourite plant genus of the bug) were obtained for the trials. A number of clean plants of *A. dealbata* in pots of 200 c.c. capacity were treated with various chemicals to test their endurance.

This being essentially an economic problem attempts were made to discover a substance which is:

(1) harmless to man and plants;
(2) conveniently applied to the roots of plants;
(3) readily obtainable at a reasonable price.

Different methods of application were employed according to the nature of the substances tested. To apply the following the plant in each case was carefully removed from the pot with its ball of soil and 2 c.c. of the liquid to be tested was injected into the soil with a hypodermic syringe at two opposite points about two inches from the upper surface of the soil.

Twenty-three of the thirty treatments killed the plant.

I. *Effect of substances on the host plants.*

1. *Substances harmless to the plants:*

Acetone	Petroleum
Carbolic acid (gave severe check)	Special fuel oil
Methyl alcohol	Terpineol
Motor spirit	

2. *Substances which killed the plants:*

Amyl acetate	Dichlorethylene	Petroleum ether
Amyl alcohol	Ethyl acetate	Petroleum and K.C.N.
Amyl nitrite	Formalin, 10 %	Tetrachlorethane
Benzaldehyde	Mandelic nitrile	Trichlorethylene
Benzene	Monobrombenzene	Vermijelli
Carbon bisulphide	Pentachlorethane	White spirit
Clove oil	Perchlorethylene	Xylol
Cresol, 10 %		

With the following substances the soil was saturated with 20 c.c. of a 10 per cent. solution or emulsion, in water. Six of the twelve proved fatal to the plant.

1. *Substances harmless to the plants:*

Amyl alcohol in tetrachlorethane	Paraffin jelly
Miscible oil	Potassium sulphocarbonate
Nicotine, 1 %	Tetrachlorethane

2. *Substances which killed the plants:*

Dichlorbenzene	Mercuric cyanide in 90 % alcohol
Iodoform	Potassium cyanide
90 % menthol in 90 % alcohol	Thymol in 90 % alcohol

II. *Effect of the substances on the insects.*

The results of the above experiments narrowed the range of possible treatments. The actions of a few, whose effect on the plant was harmless, were tested on infected plants with the following results:

Of the following 2 c.c. of liquid were injected into the soil.

Substance used	*Result*
Acetone	Killed mealy bug
Methyl alcohol	,,
Miscible fusel oil	Ineffective
Petroleum	Mealy bug escaped
Special fuel oil	,,

The plants were watered with a solution or emulsion in water of the following. All the treatments killed the mealy bug and did no damage to the plant.

Substances used:

Aniline hydrochloride	2 %	Monochlornaphthalene emulsion	5 %
Calcium thiocarbonate	5 %	Nicotine	1 %
Miscible copal oil	1 %	Potassium sulpho-carbonate	1 %
Miscible 241 oil	1 %	Pyridine	1 %
Miscible tetrachlorethane	1 %	Pyroligneous acid	5 %
Miscible trichlorethylene	1 %	Sodium sulpho-carbonate	5 %

With the above selected remedies the following trials were made at the Chelsea Physic Garden on cool greenhouse plants. A large selection of plants was treated to ascertain whether *Acacia dealbata* possessed peculiar resistance. The substance was applied by filling the pot to the brim as in the ordinary method of watering pot plants.

Substance used	*Plants*
1. Preparation of tetrachlorethane (Westoran), 1 %	*Selaginella Martensii* *Acacia platyptera* *A. spiralis* *A. cordata* *A. longifolia lineata* *A. dealbata*
2. Trichlorethylene emulsion, 1 %	*Adiantum Capillus-Veneris* *Acacias* as above
3. Pyridine, 1 %	*Erica melanthera* *Abutilon* sp. *Acacias* as above, omitting *platyptera*
4. Potassium sulpho-carbonate, 1 %	*Pelargonium quercifolium* *Abutilon* sp. *Acacias* as before

All the plants survived the treatments and the bugs were killed in every case.

The following were injected into the soil of pot plants, in the quantities indicated, with successful results, both as regards the plants and the pest (*Ripersia* sp.):

Aniline HCl, ·4 gm.
Copal oil, 2 c.c.
$CaCS_3$, 20 % solution, 2 c.c.
Chloroform, 2 c.c.
Clayton's fluid, 2 c.c.
Dichlorethylene, 2 c.c.
Ethyl formate, 2 c.c.
K_2CS_3, 40 % solution, 2, 1·5, 0·8, 0·6, 0·4 c.c.
Monobrombenzene, 2 c.c.
Monochlornaphthalene emulsion, ·2 c.c.
Miscible oil and amyl alcohol, 5 %, 2 c.c.
Naphthalene emulsion, ·2 gm.
Nicotine, ·04, ·02, ·15 c.c.
Pseudocumol, 2 c.c.
Rosin soap, ·4 c.c.
Soft soap, 2 gm.
Toluene, 2 c.c.
Trichlorethylene, 2 c.c.
241 oil, 2 c.c.
Vermijelli, 2 c.c.
Westropol, 4 c.c.

SUMMARY.

Of the list of possible remedies the following are the least desirable for using on a large scale for the reasons stated.

Nicotine	Uncertain in its action and being poisonous must be used cautiously.

Pyridine	Highly offensive odour.
Petroleum	Inflammable. Bugs dislike it, they often leave the pots and under certain conditions they may escape death.
Methyl alcohol	Inflammable, action slow.
Acetone	Will not mix with water—injections into the soil are impracticable.
Fuel oil	Bugs escape from the pots.
Miscible fusel oil	No effect.

The three following stand out as being the most effective and suitable:

Tetrachlorethane (Westoran)
Trichlorethylene (Westropol)
Potassium sulpho-carbonate

The root mealy bug and root aphis living under similar conditions in pots, adopting similar methods of feeding and protection, respond similarly to any particular treatment. The grower may, therefore, cope with both pests by the application of a common remedy.

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(*Received March 23rd,* 1926.)

ON THE MICROSCOPIC EXAMINATION OF BEES FOR ACARI

By D. MORLAND, M.A.
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(With 2 Text-figures.)

Owing to the discovery by Rennie and his collaborators (3) of the mite which causes one form of adult bee disease, it has become necessary to have a quick method of dissection in order to detect the presence of the parasite readily. The most rapid and satisfactory results are obtained when one has the use of a good binocular microscope giving an erect image. The dissection itself, however, can be carried out under a good lens. In this case, except where there is very heavy infestation accompanied by bronzing of the tubes, it will be necessary to transfer the tracheae—which are easily removed—to a slide and examine them under a compound microscope (2, 4).

The tools required are as follows:

A triangular (sailmakers') needle set in a handle.

A pair of fine sheet steel forceps, sharpened to a point.

A fine scalpel (or lancet such as is used for operations on the eye).

The bee is chloroformed or killed by crushing the head with the forceps. It is then placed on its back, and the triangular needle thrust into the square formed by the coxal joints of the second and third pairs of legs (Fig. 1, *A*, *B*). With this needle it can be pinned to a piece of cork, leather or linoleum (6). The triangular shape of the needle prevents the insect from rotating and, being fairly large, it stretches the tissues for dissection. Having fixed the bee thus, first remove the head, then remove the front legs by placing the point of the forceps under the coxal joint and lifting upwards. There remains a chitinous ring, very narrow in front and having a flap at each side covering the first thoracic spiracle. This represents the prothorax (shaded area, Fig. 1). With the scalpel, or the sharpened point of the forceps, work round this ring until it comes away easily, taking care not to damage the trachea lying behind the flaps, as it is most important to examine this region intact. The whole of that portion of the thoracic tracheal system which is liable

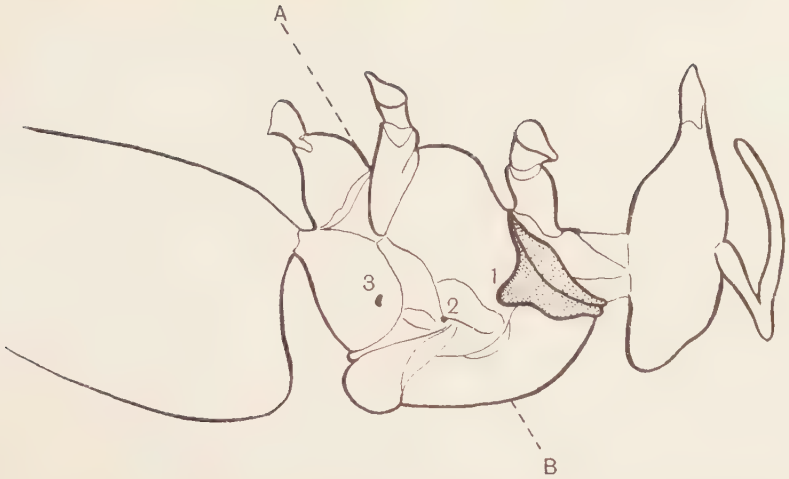

Fig. 1. Bee laid on its back for dissection. *A*, *B*, position of triangular needle for holding it steady; 1, 2, 3, thoracic spiracles (1 is situated behind the projecting flap of the prothorax). The shaded area indicates the chitinous ring (prothoracic scutum and scutellum) which has to be removed. (Adapted from Snodgrass.)

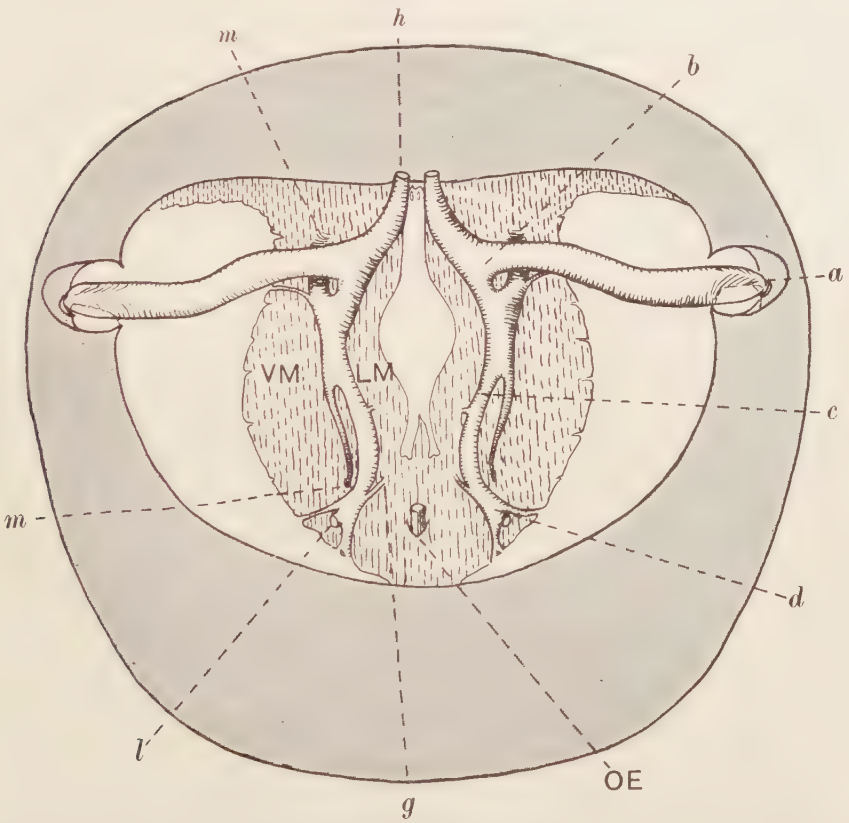

Fig. 2. Thoracic tracheal system of bee laid open for diagnosis of acarine disease. *a*, *b*, *c*, *d*, see text; *h*, branch to head; *m*, branches to air sac between muscles of flight; *g*, to salivary gland; *l*, to front leg; *OE*, oesophagus; *VM* and *LM*, vertical and longitudinal flight muscles. The white areas are air sacs.

to invasion by the mite now lies open to view (5). The mites show up as oval translucent areas in the tracheal tube while any discoloration of the trachea is easily seen (Fig. 2).

In the case of primary invasion a single female mite will be found at the point *a*, Fig. 2, and, should no mites be visible, it may be well to pull the trachea outwards to expose the other (posterior) side, as the mite will often be found showing as a translucent spot in that position.

As the bees are examined their condition is noted down in the following manner:

Hive A 2	1 2 2 1 2	1 1 0 1	
May 4 (crawlers)	*a b b a c*	*b b a*	$\frac{8}{9}$
	0 *a c b d*	0 *a a*	

Each column of three symbols represents a bee. The top line shows whether one or both sides are invaded. Should the bee be healthy figure 0 is put down to record the fact.

The second line gives the degree of infestation; thus *a* indicates the initial stage, a single female mite or one mite and two or three eggs, just at the entrance; *b* represents invasion as far as the first fork; *c* a full infection extending down the vertical tube; and *d* an exceptionally heavy invasion.

The third line gives the discoloration of the tube, this being some indication of the age of the infestation. Thus *a* is a slight tint or a few streaks; *b* a medium bronze; *c* a dark brown; and *d* a very dark tube.

$\frac{1}{0}$ $\frac{2}{a}$

In the example *a* is a single female mite on one side only, *b* represents invasion as far as the fork on each side, with slight bronzing only. Heavy invasion with only slight bronzing would lead one to suspect infection of recent origin but making active progress. Slight infestation with dark bronzing would suggest a more endemic type—disease of long standing but not making much progress. At the end of the sample, the condition can be summarised in the form of a fraction, the numerator giving the infested bees and the denominator the number examined, which is some indication of the reliance to be placed on the finding. This is perhaps better than a percentage. A vertical line after every five bees facilitates addition.

Thirty-three bees has been the customary sample. In case the mite is not found, no less number should be considered satisfactory (1). (Incidentally, it should be remembered that thirty-three bees is more than

"a matchbox full.") The writer has on two occasions found a single female mite after examining seventy bees with negative result. It is clear that a "certificate of health" cannot be given on such an examination.

The sample should be clearly indicated, whether "crawlers" or a representative sample of the population of the hive (*e.g.* "flying bees" or "bees from the combs"). For instance, if ten *crawlers* are found to be free from mites, some other cause should be looked for, such as *Nosema apis*, dysentery or starvation. On the other hand, failure to find mites in a fair sample of ten or even twenty bees from the hive is not sufficient evidence for assuming the freedom of a colony from disease.

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(*Received April 9th*, 1926.)

INSECT ATTACK AND THE INTERNAL CONDITION OF THE PLANT

By A. H. LEES, M.A.

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At various times plant pathologists with statistical leanings have made estimations of the amounts of damage caused by insects affecting crops of economic importance. These figures are usually alarmingly high and reveal a source of waste continually going on and certainly not appreciated by the ordinary man and perhaps not fully by the majority of entomologists. To anyone, however, of an enquiring type of mind the question may well present another aspect. Knowing the powers of multiplication of insects in general and the great accessibility of food plants under modern conditions of mass production one may well wonder, not so much that loss is caused as why any food plants succeed at all in producing crops. Certain checks to the multiplication of these destructive insects are of course already well known and form the basis of text-book information. Thus one may enumerate weather conditions, insect enemies, other animal enemies, fungi, hereditary resistance and so on. All these are natural checks and make up part of the grand complex known as "the balance of nature."

Under modern conditions the direct action of man is often an important factor. This action may be exerted in various ways, such as by spraying, by trap crops, by mechanical barriers and by cultural methods. There still remains, however, one factor to which perhaps not sufficient attention has been paid in the past, namely the varying endurance of the host plant. The word endurance is used in the sense originated by Dr Butler, who has treated the mycological aspects of this subject in an exhaustive manner in his book (4). He distinguishes clearly between "resistance" which is the outcome of hereditary composition and "endurance" which is a variable quality depending on external conditions. Nevertheless it is perhaps legitimate to view both states as having this in common, that they are both an expression of the internal

condition of the plant whether brought about by inherited characters or external conditions.

Natural resistance varies, as is well known, within wide limits. Thus the beech coccus, *Cryptococcus fagi* is confined to the beech and is unable to infect other trees. Other trees are said to have a natural resistance to it but no real reason for it has ever been forthcoming. The situation is simply accepted as a fact but an acceptance is not an explanation. There is no doubt a reason but it is as yet unknown. Not all insects are so specialised to one host plant. All variations can be found through insects having two alternate host plants to those, like the gipsy moth, which have a very wide range of hosts. This natural resistance on the part of the host plants is accepted perhaps too readily as an axiom, the more readily since, even were the reason known, no modification could be induced in a susceptible plant in the required direction. Only by means of breeding from resistant varieties in order that desirable characters could be added to insect resistance does there appear a way out. The process, however, is a lengthy one and but few satisfactory results have been obtained so far by direct breeding. It is, however, to the rather narrower, but perhaps more hopeful aspect of host endurance that more investigation might be usefully directed. A concrete case will perhaps at this point serve to crystallise the argument.

Plum trees in commercial plantations are frequently attacked by a bark borer, *Scolytus pruni*. This insect bores into the bark, where it lays its eggs. The developing larvae make further extensive tunnels in which they pupate. Here they spend the winter, emerging through the shot holes in the following April. The recommendation is usually made to destroy all such trees in the winter before the beetles have time to emerge. This method is no doubt based on the supposition that emerging females will fly to other neighbouring trees which they will attack in like manner. Anyone who investigates these cases in the field usually discovers that the recommendation of grubbing and burning is very seldom performed. During five years, when working in a plum district, the writer recollects no case where the recommendation has been followed. There appeared therefore no valid reason why the whole of the plum district concerned should not have been riddled with this insect. Nevertheless no such thing occurred. In this case the usual checks cited earlier in this paper did not seem sufficient. Furthermore, when the distribution of the pest was scrutinised it was found that infected trees were spasmodic in distribution and usually isolated and that there was no sign of distribution starting from a single infected tree. The situation

evidently called for some other explanation than the one usually given. Closer observation showed that in the vast majority of cases at any rate attacked trees had been checked in some other way before the *Scolytus* had succeeded in obtaining access. The check in most cases was caused by, or associated with, an attack of a fungus producing the condition known as "die-back" (3).

In the few cases where no "die-back" disease could be seen the necessary preliminary check was caused by either hoe or rabbit damage. In other words the tree had been checked by a partial ringing. It appeared to be difficult to avoid the conclusion that a tree was not susceptible to *Scolytus* attack until it had been checked in some way. This hypothesis would at any rate explain why the attack was so often confined to single trees and not to groups of them. This case can be paralleled in the case of tea and rubber. It has been suggested that the reason of non-success of the insect in healthy trees is that so much sap is exuded from the bored healthy tree that the insect is drowned in it.

Whatever the explanation, the facts suggest a very interesting line of investigation. No longer is it a matter of unalterable natural resistance, a fixed constant so to speak, but the resistance seems to be variable according to the condition of the plant. In this particular case the resistance could be lowered by direct human action, *e.g.* by over-ringing. This appeared to the writer to be a matter of great importance since if by man's direct action host resistance can be modified in a downward direction it should follow that contrary treatment would modify it in the upward.

It has been the writer's endeavour to collect cases where host plants have been modified in insect endurance either in an upward or downward direction. Their number is but few and the writer makes no claim for completion. No doubt there are others but such as there are show clearly that a fertile field for research lies open. Needless to say such a field would have an intensely practical bearing. Just as in medicine, charms and quackery have lost their hold and even drugs are looked at somewhat askance, while right methods of living and preventive medicine are favoured, so perhaps, in the future more reliance will be put on correct cultural conditions than on spraying, and the conditions of the host plant be more closely watched than the presence of the insect parasite.

Case 1. *Effect of irrigation on berseem* (Trifolium alexandrinum) *aphis in Egypt.* It is a well-known fact that the more heavily irrigated fields

and also the lower-lying fields are much more affected by aphis attack than fields under the opposite conditions. The plants make a more lush growth and presumably the aphis can multiply faster.

Case 2. *Effect of irrigation on almond and peach aphis at Pusa.* Howard (9) states that heavy winter or spring irrigation very greatly increased the amount of aphis damage. So serious was this that, though satisfactory from other points of view, the method had to be given up on this account. With a last irrigation done in the previous October no increased susceptibility occurred. He lays great stress on the connection between soil aeration, plant growth and insect attack, and refers the evil effect of over-watering more to lack of soil aeration than to excessive water.

Case 3. *Effect of damage to roots by red bug* (Dysdercus cingulatus) *on* Hibiscus *at Pusa.* Howard (9) states that the red cotton bug on *Hibiscus cannabinus* at Pusa also follows the destruction of fine roots. This appears to occur in September and October and at that time swarms of red bug appear on the plants which, in the earlier rains, have been free. It is also a remarkable fact that the wilt-free plots at the Station remain free from red bug.

Case 4. *Susceptibility of aphis by bearing and non-bearing apple trees.* A Herefordshire grower called attention to the fact that in spring apples bearing a crop are much more seriously attacked by aphis than those not bearing a crop. The same relationship has been observed elsewhere in the case of plums.

Case 5. *The greater resistance to aphis of grass orchards compared with arable.* There appears to be a very general opinion that grass orchards in this country are less attacked by aphis than orchards of the same kind of tree in arable land.

This undoubtedly applies to apples and plums but the reason is still obscure. It may be compared with the fact that high stimulation with nitrogen appears to give a lowered resistance also. In both cases, 4 and 5, lowered resistance is associated with a lush growth and the connection certainly suggests a water factor rather than a manuring one. There is, however, so little definite information on the point that discussion is at present premature. It suggests, however, a very interesting line for investigation.

Case 6. *Effect of dung application on leaf curling aphis* (Anuraphis prunina) *of plums.* Maltby (12) reports a very interesting result on the application of dung to Pershore egg plums in Worcestershire. The

application of 30 tons of dung in autumn resulted in a greater susceptibility to leaf curling aphis on these trees two springs later. The experiment was repeated the following year with identical results in the second spring.

In both cases a full growing season intervened before increased susceptibility occurred. It is difficult, however, to come to any definite conclusion as to whether the effect was due to high feeding or to increased water-holding power of the soil coupled perhaps with greater root development.

Case 7. *The effect of rainfall on the production of big bud in the black currant variety "Seabrook's Black."* This variety is (11) highly resistant to big bud attack in the drier easterly part of England (14) where the average rainfall is 11·51 for the growing months April to September (annual rainfall 23·02, Chelmsford).

When grown in the Midlands its resistance is distinctly lowered with an April-September rainfall of 12·72 (annual rainfall 24·07, Evesham).

When grown in the wet south-west it becomes comparatively susceptible in an April-September rainfall of 16·50 (annual rainfall 34·88, Clifton).

This susceptibility seems in direct line with the water at the roots during the growing season and also with the fact that a cut-down black currant appears more susceptible than an uncut-down one. The cutting down alters the ratio of top to roots and causes the relatively succulent growth which in other cases has been found peculiarly susceptible to insect attack.

Case 8. *The effect of rains on a* Psylla *attacking indigo.* Howard (9) states that indigo is attacked in India by a *Psylla* only during the rainy season. When the rains cease the attack also ceases. He further states that an application of undecayed organic manure or an oil-cake dressing produce a *Psylla* attack. He explains this by stating that associated with these treatments there is a restricted development of active roots.

It has been found in apples (2) and also in strawberries (13) that the greatest root development takes place when the shoot growth has already slowed down at the end of the growing season and that during strong shoot development root growth is slight in amount.

It may be suggested therefore that the poor root development of indigo during the rain is but an accompanying effect of high rainfall and not the true cause of the *Psylla* attack, which might be associated with succulent condition of the above ground portion caused by high rainfall or organic manure.

A comparative case occurred in a private garden where a cordon apple, cut back hard to eliminate canker, became especially susceptible to aphis while its uncut neighbours, whose summer shoots intermingled, remained practically free.

Case 9. *The relative susceptibility of healthy and unhealthy plum trees to Scolytid attack.* This case is one of the comparatively numerous cases that appear to be known of the susceptibility of trees checked by some other cause than insect attack. In this particular case, discussed previously in this paper, the checking factors suggested were "die-back," rabbit damage and hoe injury.

Thus Osterwalder(16) found a prune tree infested by *S. rugulosus* with the lower portion attacked by *Fusarium* sp., *Clasterosporium* sp. and an unidentified species. "Injury by the fungi had apparently encouraged the bark beetle, and such a form of beetle infestation may prove dangerous to neighbouring trees that are free from fungi."

Schneider-Orelli(17) in referring to *Xyleborus dispar* on apple and plum states that "any mechanical injury which interferes with the circulation of sap, even though only temporarily, provides the most favourable conditions for attack by this beetle. It has been erroneously assumed that very vigorous trees are chiefly attacked, but examination of the few bores in such trees shows that the work has been stopped by the flow of sap and that in cases where the oviposition has been affected the eggs rot." This is a typical statement of many writers discussing bark and wood borers and the view appears to be widely held.

Case 10. *The painted hickory borer in relation to hickory trees.* Dusham(7) calls attention to the fact that healthy hickory trees are not attacked by this Cerambycid beetle but only recently killed standing trees and felled timber with the bark still on.

Here again, as in the *Scolytus* cases, the necessary condition for attack appears to be diminution of sap supply.

Case 11. *Borers in rubber trees.* According to Wardle and Buckle(19) quoting Sharples(18) "the general opinion held by planters in the Middle East is that small boring beetles such as the Scolytid, *Xyleborus parvulus*, and the Longicorn, *Pterolophea melanura*, cannot penetrate the bark of a healthy rubber tree without being killed by the latex and only when the lactiferous cells have been killed by fungi can such beetles reach the wood."

These two writers suggest however that the real explanation may be that the resistance is due to the cork layer. In support of this they

cite the fact that if the cortical layer be injured or removed by fire after a spell of dry weather the scorched trees are quickly attacked by borers, many of which reach the wood in spite of the exuding latex. On the other hand it is at least a possible explanation that fire injury would inhibit latex exudation and that though some latex might be present the quantity might not be enough to prevent insect attack.

The following two cases illustrate the effect of situation and lay-out on insect attack. Both these two factors are controllable.

Case 12. *Boll weevil cotton in Texas.* Cook(6) has shown that the amount of attack by boll weevil on cotton depends very largely on temperature. If it is high enough the insect is greatly checked even inside the boll. This can be attained by spacing the cotton plants far enough apart, by which means the sun is able to exert its full powers. Close planting means shade and coolness and consequent increase of pest.

Case 13. Chamberlin(5) in describing the ravages of the western pine bark beetle, *Dendroctonus brevicornis*, states that it is favoured in regions of severe storms where lightning-struck trees are frequent or where high winds or heavy snows have broken or thrown trees. Such timber, being weakened, offers less resistance to the entering beetles.

Case 14. *The influence of potassic manure on the resistance of tea to* Helopeltis. Andrews(1) in a very interesting and now classic experiment has shown that a high rate of available potash to available phosphoric acid in the soil in which tea plants are growing causes a strong resistance to the "mosquito blight." Success however was not always obtained by adding potash manures to the soil since the plant apparently cannot always make use of the added manure. When a direct injection however was made heavily infested bushes were completely freed from the insect by this means.

Discussion.

The fourteen cases cited above all possess one common attribute, that the susceptibility to insect attack varies with the conditions under which the plant is growing. There can be little doubt that the external conditions have a direct influence on the true internal condition of the plant. Kraus and Kraybill(10) in their work on the tomato have shown clearly that the internal condition, as judged by chemical analysis, has a direct correlation with fruiting and vegetation. It does not matter for the purposes of the argument whether their methods and results are correct from a strictly chemical standpoint; they have at any rate shown that it is the internal condition of the plant that regulates its growth

and fruiting activities. In their experiments they brought about these changes in internal condition by varying such external factors as manuring, leaf area and light supply, and it is therefore a fair inference to suppose that change of external conditions will, in general, have its reflex in change in internal make up. It is thus legitimate to conclude that in the cases cited in this paper such operations as irrigation and manuring cause a *pari passu* change in the internal state of the plant. These changes must of course have their influence on the attacking insect though at the present time, with the dearth of necessary physiological and entomological data, it is impossible to ascertain their exact nature. Nevertheless, when dealing with insects like aphids, capable of producing many generations in a short time, it does appear as if internal water supply or nitrogen or both are favourable conditions for their success.

Thus the influence of soil moisture appears strongly in the case of berseem and peach aphids and in big bud and *Psylla* susceptibility of indigo. In the first two cases increased susceptibility results from over-irrigation, in the second two, from excessive rainfall. Apparently closely allied to this condition is the greater aphis susceptibility of apples on arable land compared with those in grass, as well as the similar increased susceptibility of a cut-back apple quoted under Case 8. It is difficult to hazard an explanation of the connection between aphis attack and bearing apples and plums against the opposite condition of non-bearing and comparative freedom, but the work of Hooker, Harvey, Murneek and others is suggestive.

Hooker(8) showed that in the apple the carbohydrate-nitrogen relations were associated directly with the process of flower formation. At the time of flower bud differentiation, of which the first visible sign is seen usually some time in July, non-bearing spurs, namely those that are forming flower buds to bear next year, had less total nitrogen and reducing sugars and more starch and hydrolysable polysaccharides. The carbohydrate-nitrogen ratio is in other words high in spurs developing flower buds.

At the time of setting, however, in the following spring Hooker found a tendency for bearing apple spurs, namely the non-bearers of the previous year, to contain a maximum of nitrogen. Murneek and Harvey(15) also showed that setting in the apple was correlated with low values of the carbohydrate-nitrogen ratio but with the same ratio, if nitrogen is decreased, fruiting decreases. All these writers agree therefore in the fact that for setting in the apple nitrogen must be present in sufficient

quantity in the spur and that a low C/N ratio is not enough. Probably the same argument would apply to plums and the susceptibility to aphis attack may again be brought into line with high nitrogen content. Exactly what is the effect on the aphis is not clear but the higher supply of nitrogenous food is not unlikely to give a higher reproductive power to the insect.

The direct effect of manure is well illustrated by the classic case of *Helopeltis*, where a definite potash to phosphoric acid ratio was obtained. In this case the question of multiplication rate does not enter and the explanation seems to be that the insect actually objects to the taste, since Andrews mentions that treated plants are sampled but that the attack is not repeated.

Whether the effect of dung on plum aphis is a direct manurial one is not ascertainable. The increased water holding power of the dunged soil and the known greater root development under these conditions may bring the plant into the same susceptible condition as has been found elsewhere to follow irrigation.

The cases of bark and wood borers, whether in plum, rubber or hickory, all seem to depend on conditions hindering the proper sap flow. Once however such a pest nursery is established in injured trees there is a tendency for the attack to pass to healthy trees, probably owing to mass infection.

Attempts at explanations of observed facts such as the above, though they may give indications of conditions that should be secured in the field, leave much to be desired from the scientific point of view.

In view however of the work that has been and is being done on the relations of internal conditions to plant growth, the time would appear to be ripe for similar investigations on the relationship of internal conditions to insect endurance. That there is a definite relationship in some cases appears to be shown by the examples cited in this paper. How far similar relationships apply in other cases of insect attack it is of course impossible to say. Certainly many cases occur to the mind in which there is apparently no connection. It is however perhaps premature to come to any conclusion even in these cases until many more facts can be collected. The whole subject calls for close collaboration between entomologists and biochemists. To suggest that there is a profitable field for such work if initiated on sound lines and perseveringly carried through is the object of this paper.

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(*Received March 11th,* 1926.)

INFLUENCE OF PROTOZOA ON THE PROCESS OF NITROGEN FIXATION BY *AZOTOBACTER CHROOCOCCUM*

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(With Plate XIV and 3 Text-figures.)

Introduction.

Since Russell and Hutchinson (19) put forward the well-known theory of the possible relationship of protozoa to partial sterilisation of soils, various workers have attacked this postulation from different angles. For instance, in the United States it has been maintained that protozoa were normally present in the soil largely, if not exclusively, as cysts and that they could not therefore influence the bacterial activities to any appreciable extent. Such a view, however, was shown to be incorrect by Martin and Lewin (13) who demonstrated that in Rothamsted soils there exists a trophic fauna: a view later confirmed by Waksman (21) for American soils. Later investigations by Cutler (5) and his associates in England and by Perey (18) in France gave additional proof of this activity of protozoa. The fact that they limit the numbers of bacteria has also been shown by the work of Cutler, Crump and Sandon (6) in which daily counts, extending over a period of one year using the newly devised and accurate methods, were made.

The attention of various workers was thus drawn towards the question of the possible influence of protozoa on the process of ammonification in the soil, but practically nothing has been done to test their influence on other equally important biological processes like nitrification and nitrogen fixation. Hills (10) as a result of certain experiments concluded that nitrogen fixation was increased by the presence of protozoa in soil cultures, but in liquid cultures he could find no evidence for such an effect. In the year 1923 Nasir (16) made preliminary experiments with a view to observing if there was any influence of protozoa on the process of nitrogen fixation by *Azotobacter*. His results indicated

that generally speaking *Azotobacter* fixed, during a period of 15 days' incubation, more nitrogen in the presence of protozoa than in their absence.

Nasir was unable to continue the work and when one of us came to this department for post-graduate research this problem was suggested to him, since it was thought that a critical study was necessary to understand the exact significance of this process[1].

Methods employed.

(*a*) *Media.*

The composition of the mannitol agar and solution used in the experiments was as follows:

KH_2PO_4 0·2 gm. (made alkaline before		NaCl	0·2 gm.
the addition of other ingredients using		$CaSO_4$	0·1 ,,
phenolphthalein as indicator)		$CaCo_3$	5·0 ,,
$MgSO_4$ 0·2 gm.		Water	1000 c.c.

For liquid cultures the above solution with the requisite amounts of mannitol was employed, except in the experiments (Table V) where calcium carbonate was omitted. The solid media were prepared by adding 20 gm. of agar and the required quantity of mannitol to the above solution.

(*b*) *Nitrogen estimation.*

For estimating nitrogen in solid and liquid media the usual Kjeldahl method was employed, but for the estimation of nitrogen in soil the modified "wet" method described by one of us (1) was employed. In all cases $N/10$ acid and alkali was used and a difference of 0·5 c.c. or more of acid was regarded as significant.

(*c*) *Mannitol estimation.*

The method used for the estimation of mannitol was that described by Christensen (3) and further modified by Christensen and Bondroff (4).

(*d*) *Method of making bacterial and protozoal counts.*

The haemacytometer method described by one of us (7) was employed for making the counts of bacteria and protozoa. For

[1] The problem was developed and the experiments were made by D. V. Bal and the interpretation of the results is a conjoint production. Mr Bal takes this opportunity to express his gratitude to Sir John Russell for having allowed him to work at this station. He is also indebted to the Local Administration, Central Provinces, India, and to Mr F. J. Plymen and Dr H. E. Annett for making the visit to Rothamsted possible.

counting on solid culture media the whole growth was very carefully removed, washed into graduated cylinders and made up to a known volume. After thoroughly mixing the emulsion it was then employed for making the counts. The counts from each culture were done separately in duplicate or triplicate and the average was taken as the representative figure. For each bacterial count 45 squares were observed and for each protozoal count the total number of organisms in the whole chamber or in a requisite number of squares was counted, according to the density of the population, since the error depends on the square root of the number of organisms counted.

(*e*) *Protozoan cultures.*

The protozoa used were confined to two species, one a ciliate, *Colpidium colpoda*, and the second an amoeba, *Hartmanella hyalina*, one of the commonest soil protozoa.

Colpidium colpoda is also a soil form, but is by no means as common as the amoeba, it was used in the experiments because so much work has already been done with it in this department (7, 8) that its cultural peculiarities are now well known and easily controlled.

Experimental Results.

In the first instance experiments, as conducted by Nasir, were repeated; and in addition experiments in combined solid and liquid media according to Ashby's method were carried out. Great difficulty was encountered in obtaining cultures of protozoa on mannitol agar, since the stock cultures were growing on nutrient agar. After repeated efforts it was found, however, that they soon got acclimatised and began to grow profusely in the required media. Two pure strains of *Azotobacter chroococcum* were used, one isolated from the Barnfield soil and the other from the black cotton soil of India. The bacteria growing with the protozoal cultures were isolated and used where necessary for inoculation with the *Azotobacter*, so that any possible effect of association with such bacteria was eliminated. In the tables the phrase "other bacteria" refers to these organisms. This was done to ensure that any increased fixation in the presence of protozoa could not be ascribed to the associated bacteria. The early experiments were carried out using Ashby's mannitol agar containing 20 gm. of mannitol per litre. Quantities of 50 c.c. containing 1 gm. mannitol were sterilised, made into slopes in 300 c.c. Erlenmeyer flasks and inoculated with the appropriate cultures in such a way that the same numbers of *Azotobacter* were added in all

cases. To the cultures containing *Azotobacter* alone sufficient sterile water was added to bring the quantity of fluid to the same amount as in the flasks inoculated with *Azotobacter* and protozoa.

In order to keep the initial nitrogen content of the flasks constant the control set also received the appropriate amount of inoculum, after which they were immediately sterilised.

The results obtained are given in Table I.

Table I.

Azotobacter *strain from Barnfield.*

Nitrogen fixation by Ashby's method (combined solid and liquid medium).
100 c.c. solution containing 1 gm. mannitol.

	Milligrams of nitrogen fixed after 15 days
Azotobacter and other bacteria	2·5, 2·65 } 2·57
Azotobacter, *Colpidium* and other bacteria	3·68, 4·41, 3·38 } 3·82

Solid medium containing 1 gm. mannitol per culture.

	Milligrams of nitrogen fixed after 15 days	
	Series 1	Series 2
Azotobacter and other bacteria	3·24, 3·60 } 3·42	3·9, 3·75 } 3·82
Azotobacter, *Colpidium* and other bacteria	2·94, 3·38 } 3·16	3·9, 3·83, 4·04, 4·27, 4·19 } 4·05
Colpidium and other bacteria	Nil, Nil	—, —

Azotobacter *strain from black cotton soil, India.*

	Milligrams of nitrogen fixed after 15 days		
	Series 3	Series 4	Series 5
Azotobacter and other bacteria	3·02, 2·87 } 2·94	4·27, 4·12 } 4·19	3·68, 3·38 } 3·53
Azotobacter, *Colpidium* and other bacteria	3·75, 3·90 } 3·82	4·4, 4·12 } 4·26	—
Azotobacter and *Hartmanella*	—, —	—, —	3·82, 3·24 } 3·53

It will be noticed that increased nitrogen fixation does not always occur in the presence of protozoa. This led to further experiments designed to discover the reasons for this disparity; and the effect of 0·25 and 0·5 gm. of mannitol in the same quantity of media as before was tested.

The results given in Table II show that with low quantities of mannitol, *e.g.* 0·25 gm. instead of 1 gm., increased nitrogen fixation always takes place and the proportionate percentage increase is greater in the lower concentrations. Also in pure cultures the amount of nitrogen fixed per gram of mannitol is proportionately greater in low concentrations (11). Similar experiments using liquid mannitol solution were also made, the results of which (Table II, Section B) were in accordance with those given for solid media.

Table II.

Fixation of nitrogen, after a period of 15 *days' incubation, in solid and liquid media with varying quantities of mannitol.* Azotobacter *strains from black cotton soil.*

Section A. Solid Media.

50 c.c. medium containing 0·25 gm. mannitol per culture.

	Series 1		Series 2		Series 3		Series 4	
	Milligrams of nitrogen fixed	% Increase	Milligrams of nitrogen fixed	% Increase	Milligrams of nitrogen fixed	% Increase	Milligrams of nitrogen fixed	% Increase
Azotobacter and other bacteria	1·03 1·03 } 1·03	—	0·88 1·03 } 0·95	—	1·18 1·03 } 1·10	—	0·735 0·883 } 0·81	—
Azot.,*Colpid.* and other bacteria	2·35 2·50 } 2·42	135	2·50 2·35 } 2·42	155	—	—	—	—
Azot., *Hart.* and other bacteria	—	—	—	—	2·21 2·21 } 2·21	100	1·91 1·47 } 1·69	108

50 c.c. medium containing 0·5 gm. mannitol per culture.

	Milligrams of nitrogen fixed	% Increase
Azotobacter and other bacteria	2·06 2·65 } 2·35	—
Azotobacter, *Colpidium* and other bacteria	3·40 3·09 } 3·24	38

Section B. Liquid Media.

	100 c.c. medium containing 0·25 gm. mannitol per culture		100 c.c. medium containing 0·5 gm. mannitol per culture	
	Milligrams of nitrogen fixed	% Increase	Milligrams of nitrogen fixed	% Increase
Azotobacter and other bacteria	1·18 1·18 } 1·18	—	1·18 1·32 } 1·25	—
Azotobacter, *Colpidium* and other bacteria	2·65 2·50 } 2·57	118	2·21 2·65 1·91 2·06 2·21 2·50 } 2·25	80

It has been observed by various workers, *e.g.* Omeliansky (17), Koch and Seydels (12), and Mockeridge (14), that the nitrogen fixed by *Azotobacter* is greater in the first stages of its growth. According to Koch and Seydels this indicates that in the later stages of fixation, when there occurs an accumulation of nitrogenous material in the medium, the organisms employ the carbohydrates for purposes other than nitrogen fixation. Mockeridge (14) thinks that there is a decrease in the efficiency of the organisms due to the accumulation of their metabolic products, while Golding (9) has shown that there is increased nitrogen fixation by *Bacillus radicicola* when the organisms are grown under conditions allowing the removal of such products. It was possible therefore that the increased nitrogen fixation by *Azotobacter* in the presence of protozoa might be due to the removal by the latter of some of the metabolic products formed by the former. Consequently an experiment was devised in which the metabolic products and the surface bacterial growth were removed at frequent intervals and the nitrogen fixed was compared with that from cultures growing in the normal way. For this purpose cultures containing 50 c.c. of agar medium with 1 gm. mannitol were inoculated with equal numbers of *Azotobacter* and treated as shown in Table III.

Table III.

No.	Treatment	Milligrams of nitrogen fixed after 20 days	
1, 2	Growth on the surface agar washed every 6th day with sterile water and the washings accumulated on the bottom of the flask	3·82 3·97	3·89
3, 4	Growth washed as above but the washings were removed to Kjeldahl flasks	3·60 3·53	3·56
5, 6	Usual method without washings	4·12 4·04	4·08

This experiment, though simple in character, would indicate that the removal of growth products does not maintain at the highest possible level the efficiency of the organism.

After the demonstration that increased nitrogen fixation in the presence of protozoa was more readily obtained in low concentrations of mannitol, it was decided to study the course of mannitol decomposition, nitrogen fixation and the relative development of the *Azotobacter* and protozoa in the cultures. This was done in liquid media containing 0·25 and 0·5 gm. of mannitol per 100 c.c. The flasks were inoculated with 0·46 million of *Azotobacter* per c.c. and 532 *Colpidia* per c.c. where necessary. Incubation was continued for 15 days. The results obtained are given in Table IV, from which it is seen that the amount of nitrogen

fixation is the same as was previously found both in solid and liquid media (Table II). It is of interest that the amount of nitrogen fixed is proportional to the amount of mannitol decomposed, but the numbers of *Azotobacter* are less when protozoa are present although the nitrogen fixed and mannitol decomposed is greater.

Table IV.

Amount of mannitol decomposed and the relative growth of Azotobacter *and protozoa after* 15 *days' incubation.*

	Mannitol decomposed in gm.	*Azotobacter* in millions per c.c.	*Colpidia* per c.c.	Nitrogen fixed	% Increase
Medium with 0·25 gm. of mannitol.					
Azotobacter and other bacteria	0·152, 0·148 — 0·15	55·1, 55·5 — 55·3	Nil	1·18, 1·18 — 1·18	—
Azot., Colpid. and other bacteria	0·300, 0·271 — 0·285	51·1, 40·0 — 45·5	31,850, 22,295 — 27,072	2·65, 2·50 — 2·57	118
Medium with 0·5 gm. of mannitol.					
Azotobacter and other bacteria	0·099, 0·134 — 0·117	66·6, 68·4 — 67·5	Nil	1·18, 1·32 — 1·25	—
Azot., Colpid. and other bacteria	0·230, 0·239 — 0·235	55·1, 54·2 — 54·6	38,220, 31,850 — 35,035	2·21, 2·65 — 2·43	94

In view of the results obtained it was decided to trace the decomposition of mannitol at different intervals during the period of incubation using 1 gm. mannitol instead of 0·25 or 0·5 gm. A set of Erlenmeyer flasks in triplicate for each treatment was inoculated according to Ashby's method. A known quantity of the solution was removed at intervals for the estimation of mannitol and the numbers of organisms from duplicate flasks of each set were counted. Nitrogen was determined in the third flask at the end of the incubation period. The results obtained confirmed the previous observation of the reduction of *Azotobacter* in the presence of *Colpidia* and the greater amount of mannitol decomposed by *Azotobacter* and *Colpidia* than by *Azotobacter* alone. Certain experimental difficulties were, however, experienced in this method due to the evaporation of the solution thereby concentrating the mannitol in the flasks and to the removal of certain quantities of the liquid at intervals.

To obviate these difficulties a set of 48 test tube cultures containing exactly the same amounts of mannitol solution, *e.g.* 10 c.c. of 10 per cent. mannitol solution in each tube measured by means of a burette, was inoculated with the respective organisms. The numbers of each used for inoculation were uniform for the whole set. For the determinations

whole contents of the test tubes were employed and the amount of mannitol decomposed, and the number of the organisms in each case counted. These determinations were done in duplicate and the results are given in Table V and Figs. 1, 2.

Table V.

	Azotobacter and other bacteria		*Azotobacter*, *Colpidia* and other bacteria			*Colpidia* and other bacteria	
	Mannitol decomposed in mg. per 100 c.c. solution	*Azotobacter* in millions per 10 c.c.	Mannitol decomposed in mg. per 100 c.c. solution	*Azotobacter* in millions per 10 c.c.	*Colpidia* per 10 c.c.	Mannitol decomposed in mg. per 100 c.c. solution	*Colpidia* per 10 c.c.
At start	Nil	2·26	Nil	2·26	3,246	—	3,246
After 2 days	0·9	106·7, 80·0 } 93·3	2·2	71·1, 80·0 } 75·5	19,095, 12,730 } 15,912	—	—
„ 4 „	0·5	426·7, 133·3 } 280·0	0·9	160·0, 169·0 } 164·5	15,910, 15,910 } 15,910	—	—
„ 6 „	0·5	133·3, 142·2 } 137·0	Nil	71·1, 80·0 } 75·5	22,270, 22,270 } 22,270	—	—
„ 12 „	70·0	177·8, 257·8 } 217·8	101·5	115·6, 106·7 } 111·15	280,000, 135,800 } 207,900	—	—
„ 19 „	91·0	293·4, 167·0 } 280·2	241·5	142·2, 88·9 } 115·55	381,900, 254,600 } 318,250	—	—
„ 26 „	112·0	187·0, 213·4 } 200·2	308·0	80·0, 115·6 } 97·8	140,030, 178,220 } 159,125	21·0	—

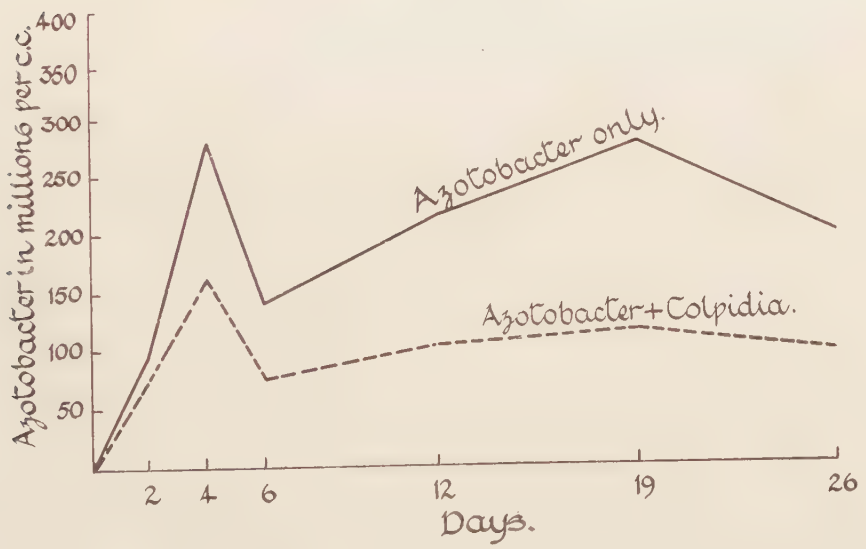


Fig. 1. Curve showing the decrease in the numbers of *Azotobacter* due to the presence of *Colpidium colpoda*.

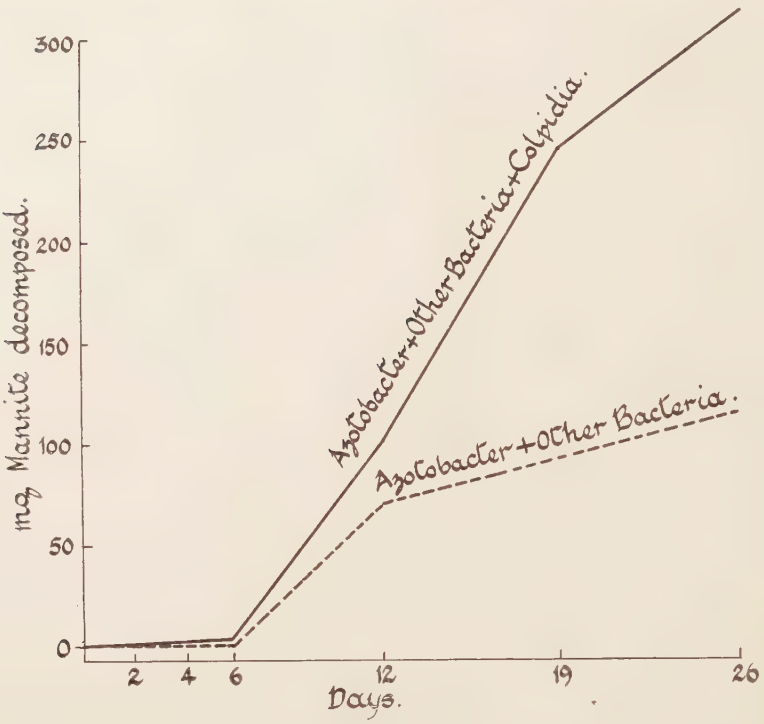


Fig. 2. Curve showing the progressive decomposition of mannitol.

It will be observed that the protozoa tend to increase the mannitol decomposition by *Azotobacter*, and that the number of *Azotobacter* is throughout lower than in those cultures where protozoa are present. It will be seen, however, from Plate XIV, fig. 3 *a* and *b*, that the total amount of growth in the cultures with protozoa is greater than when they are absent. This is further supported by the results given in Table VII. For the first week of the experiment there seems to have been a rather low activity. This was in accordance with expectation from previous experience, which showed that the organisms take some time to become established.

The following experiment was carried out to see to what degree *Colpidia* used *Azotobacter* as their food. A suspension of *Azotobacter chroococcum* in sterile tap water was heated in boiling water for about 10 minutes, cooled and the numbers of dead *Azotobacter* cells present per c.c. counted. To known amounts of this emulsion a known number of *Colpidia* was added. All the test tubes were then incubated for a period of 1 week and the numbers of cells counted. The results obtained are given below, Table VI.

Table VI.

	At beginning		After 1 week	
	Dead *Azot.* cells in millions	*Colpidia* at start	Dead *Azot.* cells in millions	*Colpidia*
Azotobacter only	193·8	Nil	190·26	Nil
Azot., *Colpid.* and other bacteria	193·8	13,494	56·01 80·00 } 68·0	55,000 59,570 } 57,285
Colpidia and other bacteria	Nil	13,494	Nil	31,700

The results definitely show that *Colpidia* feed on *Azotobacter*. Bonazzi(2) in his paper states that *Azotobacter* is incapable of either ingestion or digestion by protozoa. Our results do not support this conclusion. An experiment was carried out to see if the amoeba, *Hartmanella hyalina*, behaved in the same way. Owing, however, to the tendency of this organism to encyst on transference to the liquid suspension the results were unsatisfactory.

The effect of both *Colpidia* and *Hartmanella* on the growth of *Azotobacter* on solid media was tested by inoculating the organisms on to 50 Petri dishes each containing 50 c.c. of 0·5 per cent. mannitol agar medium and incubating for a period of 1 month. At intervals of 1, 2 and 4 weeks the growth from duplicate Petri dishes was carefully

Table VII.

	At start				After 1 week				
	Azotobacter in millions per c.c.	*Colpidia*	*Hartmanella*	Total growth in mg.	*Azotobacter* in millions per c.c.	*Colpidia*	*Hartmanella*	Total growth in mg.	Nitrogen fixed
Azotobacter and other bacteria	0·418	Nil	Nil		94·2	—	—	35	
,, ,,	,,	Nil	Nil		66·9	—	—	42	
Azotobacter, *Colpidia* and other bacteria	,,	27	Nil		61·7	15,710	—	44	
,, ,,	,,	27	Nil		63·3	35,680	—	44	
Azotobacter, *Hartmanella* and other bacteria	,,	Nil	8,214		43·02	—	200,000	53	
,, ,,	,,	Nil	8,214		38·23	—	140,000	52	

	After 2 weeks				After 1 month					
Azotobacter and other bacteria	87·1	—	—	34	79·7	—	—	28	2·85 2·92 }	2·88
,, ,,	80·0	—	—	29	76·2	—	—	33		
Azotobacter, *Colpidia* and other bacteria	61·9	17,160	—	29	60·1	—	—	37	3·52 3·80 }	3·66
,, ,,	58·0	13,120	—	33	58·3	—	—	36		
Azotobacter, *Hartmanella* and other bacteria	43·5	—	160,000	42	54·2	—	520,000	47	—	
,, ,,	56·9	—	166,700	48	63·5	—	880,000	48	—	

removed and the whole mass uniformly suspended in 100 c.c. of sterile water. The number of organisms in the respective emulsions was counted and the total amount of growth in each case in terms of dry weight was determined. The amount of nitrogen fixed was also estimated at the end of 1 month. The results are given in Table VII.

Thus it is clearly shown that the action of *Hartmanella hyalina* on the growth of *Azotobacter* is similar to that of *Colpidium colpoda*; and that the total amount of dry matter produced is greater in the protozoal cultures than in those containing only *Azotobacter*. This would appear anomalous but it is important to remember that the reduction in the bacterial numbers, as observed by microscopical examination, does not indicate that in a given period of time the total numbers of bacteria are less when protozoa are present. The counts refer only to the numbers of *Azotobacter* present when the observations are made and take no cognisance of those which have been used as food by the protozoa.

Hence, though the numbers of bacteria appear less when protozoa are present, the total growth is actually increased. According to Moler (15) the presence of protozoa, by the production of an enzyme, causes the liberation of soluble nitrogen in cultures of *Azotobacter chroococcum*. In pure culture no such nitrogen is produced by this species of bacterium, though it is by other species.

A few nitrogen estimations from pure cultures of *A. chroococcum*, containing protozoa, were made on extracts obtained by passing the emulsions through Chamberlain filters. Soluble nitrogen was found in all the cultures.

In view of the two methods commonly employed for testing the nitrogen fixing power of soils, attention was next directed towards testing the action of protozoa in liquid media, using soil as an inoculum instead of pure cultures of *Azotobacter*. Soil, to which mannitol is added, is used as the medium in one method but in the second method 1 gm. of the soil to be tested is added to liquid mannitol solutions. It was thought therefore that it would be of interest to see whether the introduction of active protozoa such as *Colpidia* would modify the course of events taking place on the addition of soil to mannitol media.

An experiment was set up in which 100 c.c. of a 1 per cent. mannitol solution was inoculated with 1 gm. of air-dried soil instead of pure *Azotobacter*. In a second set the same procedure was adopted, but *Colpidia* were also added with the soil at the beginning of the experiment. The results obtained are given in Table VIII.

Table VIII.

		Milligrams of nitrogen fixed		Mannitol decomposed in gm.
1	Mannitol solution in soil	7·21		—
2	,, ,,	7·65	7·85	—
3	,, ,,	8·68		0·895
4	Mannitol solution, soil, *Colpidia*	7·95		—
5	,, ,, ,,	7·80	8·07	—
6	,, ,, ,,	8·46		0·916

No effect was produced by the introduction of the *Colpidium*, but this is probably explained by the masking action of the numerous amoebae and flagellates developing from the cysts introduced with the air-dried soil.

Having established the fact that *Azotobacter*, growing in association with protozoa, is capable of increased nitrogen fixation in both artificial, solid and liquid media, the importance of testing whether the same obtained when using soil as a medium was realised.

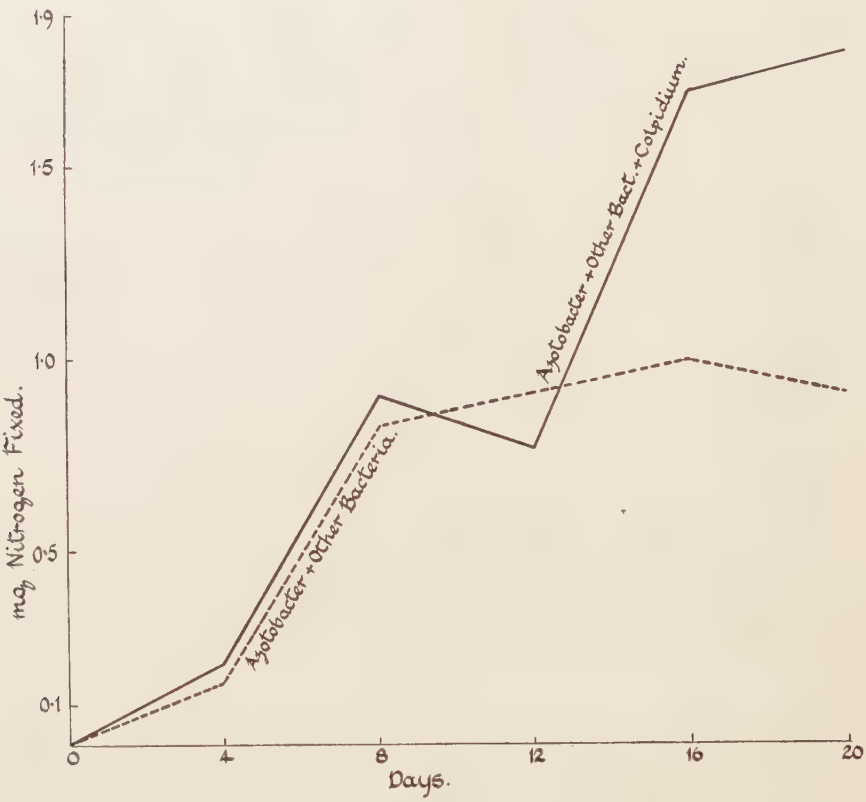
Twenty gramme portions of black cotton soil contained in Erlemeyer flasks were brought to the optimum moisture content (30 per cent.) by the addition of nutrient mannitol solution, and then sterilised in the autoclave for 1 hour on two successive days at a temperature of 125° C. The amount of mannitol added per flask was varied so that two sets of soils were prepared, one containing 0·25 gm. and the other 1 gm.

The results given in Table IX show that in soil also *Azotobacter* fixes more nitrogen when protozoa are present. Further, that in soil, as with artificial media, increased nitrogen fixation always occurs only in low concentrations of mannitol.

Table IX.

		Milligrams of nitrogen fixed after 3 weeks		% Increase
	Media containing 0·25 gm. of mannitol.			
1	Sterilised soil + *Azotobacter* + other bacteria	1·18	1·03	—
2	,, ,, ,,	0·88		
3	,, ,, + *Colpidia*	2·21	1·91	85
4	,, ,, ,,	1·62		
5	,, ,, + *Hartmanella*	1·76	1·91	85
6	,, ,, ,,	2·06		
	Media containing 1 gm. of mannitol.			
1	Sterilised soil + *Azotobacter* + other bacteria	1·76	1·83	—
2	,, ,, ,,	1·91		
3	,, ,, + *Colpidia*	2·21	2·06	—
4	,, ,, ,,	1·91		
5	,, ,, + *Hartmanella*	1·76	1·69	—
6	,, ,, ,,	1·62		

Since in all the above experiments the estimations of nitrogen were done after a definite incubation period the progress of nitrogen fixation at shorter intervals of time was followed. In Table X and Fig. 3 are given the results obtained from such an experiment where 0·25 gm. of mannitol were used.



Nitrogen fixation for shorter intervals

Fig. 3. Curve showing the progressive increase of nitrogen fixation.

It will be seen that in the early stages of incubation the differences in the amounts of nitrogen fixed is too small to be significant, but that after a period of 12 days it becomes sufficiently great to be well outside the experimental error. It is possible therefore that the 15 days' incubation period normally employed is too short to allow of maximum nitrogen fixation by *Azotobacter* and protozoa even though low concentrations of mannitol are used. That such is the case is demonstrated

Table X.

Medium containing 0·25 gm. of mannitol.

	Milligrams of nitrogen fixed			
	Azotobacter and other bacteria		*Azotobacter* + *Hartmanella* + other bacteria	
After 4 days	0·088 0·235	0·162	0·162 0·235	0·199
,, 8 ,,	0·677 0·971	0·824	0·824 0·971	0·90
,, 12 ,,	0·971 0·824	0·90	0·677 0·824	0·75
,, 16 ,,	1·12 0·824	0·972	1·71 1·63	1·67
,, 20 ,,	0·824 0·971	0·90	2·0 1·53	1·78

by Table XI, in which are given the results obtained from an experiment where 0·25 gm. of mannitol were used, but the incubation period increased to 1 month.

Table XI.

Medium containing 0·25 gm. of mannitol.

	Milligrams of nitrogen fixed		% Increase
Azotobacter + other bacteria	1·10 1·10 1·10	1·10	—
Azotobacter + other bacteria + *Hartmanella*	4·60 4·60 4·46	4·55	313

The failure to observe on every occasion increased nitrogen fixation with media containing 1 gm. of mannitol may be due to the fact that 15 days is too short a period of incubation. Further experiments were therefore begun in which cultures with 1 gm. of mannitol were incubated for 4 and 8 weeks respectively (Table XII).

Table XII.

Media containing 1 gm. of mannitol.

	Milligrams of nitrogen fixed					
	After 4 weeks		% Increase	After 8 weeks		% Increase
Azotobacter + other bacteria	1·39 1·53	1·46	—	1·98		—
Azotobacter + other bacteria + *Colpidia*	6·95 5·53	6·24	327	5·50		180
Azotobacter + other bacteria + *Hartmanella*	6·22 5·78	6·0	310	5·63 5·05	5·34	170

Increased incubation to 4 weeks has obviously materially increased the amount of nitrogen fixed in the presence of the protozoa, but a further prolongation of incubation to 8 weeks produced no additional effect. That no more nitrogen fixation occurs after a certain period with pure cultures of *Azotobacter* has frequently been observed, and has been attributed either to loss of efficiency of the bacteria or to the inhibiting effect of accumulated metabolic products; such explanations, however, are not applicable to the protozoal cultures. In these it is probable that the absence of further nitrogen fixation after the first 4-week period is due to the great development of the protozoa, which eventually become sufficiently numerous to destroy the great majority of *Azotobacter* cells.

That such is the case has been repeatedly observed, by microscopic examination of the cultures during the course of the various experiments. For the first few days numerous *Azotobacter* are found, but the numbers gradually diminish during the succeeding periods until finally at the end of 2 months it is difficult to find any cells.

A further ocular demonstration of this is afforded by the pigment-producing property of *Azotobacter chroococcum*. In a young culture the growth is raised above the surface of the slope and is mucilaginous, becoming black in colour as growth proceeds, finally flattening as the cultures become old.

When protozoa are present both the raising of the growth above the surface, and the production of pigment are reduced and in an old culture the colour entirely disappears and there is no trace of any raised growth on the slope (Plate XIV, figs. 1, 2).

Table XII also brings out the fact that the nitrogen-fixing power of the *Azotobacter* strain has diminished, for at the beginning the strain fixed from 3 to 4 mg. of nitrogen (see Table I); a passage through soil restored initial power, as is shown in Table XIII.

Table XIII.

Medium containing 1 gm. of mannitol.

	Milligrams of nitrogen fixed after 4 weeks		% Increase
Azotobacter + other bacteria	3·12 3·05	3·08	—
Azotobacter + other bacteria + *Hartmanella*	3·65 3·49	3·57	16

In this case the percentage increase is not well marked, which is in contradistinction to the first result (Table XII). This would indicate

that when the efficiency of the bacteria is increased the period of incubation should also be increased in order to show the effect of the presence of protozoa.

General Conclusions.

It will be noticed from the experiment given in Table I that the amount of nitrogen fixed in the presence of protozoa is not always in excess of that fixed by *Azotobacter* alone. This is in general agreement with Nasir's results. But it should be pointed out that the figures given by Nasir are not comparable with ours, since his figures are calculated on the basis of 1 gm. of mannitol, irrespective of the amount present in the media, whereas our figures are the actual amount of nitrogen fixed per culture. Nasir's method of giving the amount of nitrogen fixed will of necessity give higher figures than were actually obtained; because, as shown by other workers and by our own experiments the nitrogen fixed with low concentrations of mannitol are proportionally greater than when high concentrations, such as 1 gm., are employed. On comparing our results with those of Nasir, the figures shown by him in Series VII of his paper were so surprisingly high (15–24 mg. of nitrogen fixed per gram) that one of us (D. W. C.) thought that an error in transcription from the note book had occurred. On looking through the old records it was found that such was the case and that the figures should be reduced in the case where 80 c.c. media were used by the ratio of 1·6 to 1, and in the case where 800 c.c. media were used by the ratio 2 : 1. Such corrections bring our results and Nasir's into general accordance with each other.

There appears to be no doubt that in low concentrations of mannitol the presence of protozoa always induces an increased nitrogen fixation: and that this increase is paralleled by the mannitol decomposed.

It seems reasonable to assume that this increased nitrogen fixation occurs from the beginning, but that at this period the difference is of too small a magnitude to be estimated. The test used for the estimation of mannitol on the other hand being more delicate, small initial differences can very well be estimated. The fact, however, that cultures of *Azotobacter* are usually active in fixing nitrogen in the earlier period of growth, followed by a lag period (Table X) is seen from the figures of mannitol decomposed given in Table V. It will be noticed that after 6 days, when the cultures were established, the amount of mannitol decomposed was 70 mg. while in the following 2 weeks it was only 20 and 21 mg. In the cultures of *Azotobacter* and protozoa, instead

of attaining the usual lag period after 12 days, the action was more vigorous and not until the last week was any falling off observed. That protozoa take some time to get established in the initial stages and that an appreciable difference in the amount of nitrogen fixed cannot be obtained with shorter incubation period can be attributed partly to the fact that the protozoa do not reproduce rapidly until a certain concentration of bacterial numbers is attained. It has been shown(8) that the growth rate of *Colpidium colpoda* in artificial cultures is entirely dependent on the relative concentration of bacterial numbers; and a proportion of about 1,000,000 to 1 *Colpidium* was found to give the maximum rate of reproduction. The reason for the increased nitrogen fixation by *Azotobacter*, when associated with protozoa, would appear to be due to the fact that the efficiency of the organisms is maintained for a longer time than when they are growing in pure cultures. It does not follow, however, that this increasing nitrogen fixation should go on indefinitely because as already mentioned the protozoa, although they stimulate the action, or efficiency of *Azotobacter*, are continually feeding on them; and a stage comes when they grow to such an enormous extent that they almost eliminate all the *Azotobacter* from the culture. That this does not occur in soils in the field is probably due to the numerous agencies operating to preserve a balanced relationship between the various members of the soil population.

In artificial culture this balance is often upset and one or other of the associated organisms gains the upper hand, as in the case just cited. Another example of a similar nature was observed in the course of some experiments where a strain of bacteria producing yellow pigment was obtained by Dr C. E. Skinner, who was making attempts to get a pure culture of these bacteria associated with *Hartmanella hyalina*. With this culture growing on ordinary nutrient agar it was seen that in a short time the pigment forming yellow organism, which grew profusely at first, was completely eliminated by the overwhelming growth of the protozoa.

There appears to be a definite relationship between the nitrogen-fixing power of the strain of *Azotobacter* used, the incubation period employed, and the concentration of mannitol used as is shown in Table XII. With media containing 1 gm. of mannitol increased nitrogen fixation was obtained when the incubation period was lengthened to 4 weeks; but on raising the efficiency of this strain by passing it through soil it was found that the same period of incubation did not give any marked increase in nitrogen fixation (Table XIII). In view of these results it would appear that the increased nitrogen fixation in the

presence of protozoa was due to a prolongation of the efficiency of the bacteria, brought about by the protozoa keeping the numbers of bacteria at a lower level than they would attain in pure cultures. This probably results in the culture remaining in a younger and more active condition.

A secondary effect may be the removal of some of the nitrogenous waste products by the protozoa.

This increased nitrogen fixation by *Azotobacter* with protozoa at first sight appears to be contrary to the generally accepted view of the part played by protozoa in such a biological reaction of the soil as ammonification (Russell and Hutchinson (19)).

In the former the product of the reaction is contained in the bodies of the bacteria, whereas in the latter the product of the reaction (ammonia) is present in the medium; and hence there is no conservation of ammonia in the bodies of the bacteria as is the case with the nitrogen fixed by the action of *Azotobacter*.

It is also reasonable to assume that the amount of ammonia formed in the process of ammonification is mainly due to the organisms working for a certain given period and not only during the course of their active multiplication. With nitrogen fixation, however, our experiments and those of previous workers show that active nitrogen fixation takes place when the culture is young and growing. This would indicate that *Azotobacter* uses most of the energy while in the young active state of division resulting in a proportionate amount of nitrogen fixed. Thus for instance Koch and Seydels (12) hold that in the later stages of fixation, when there occurs an accumulation of nitrogenous material in the medium, the organisms employ the carbohydrates for purposes other than nitrogen fixation. The ammonifying bacteria, on the other hand, have continually to split the organic nitrogenous matter which results in the formation of ammonia as a by product. As in the case of other biological reactions this process will only go on till the concentration of ammonia attains an inhibiting point. To put it conversely it would mean that while *Azotobacter* after division no longer actively fixes nitrogen, its removal by the protozoa would only mean conversion of one form of nitrogen into the other; in the case of ammonification the organisms after their division still continue the formation of ammonia.

Summary.

1. Increased nitrogen fixation by *Azotobacter chroococcum* in the presence of protozoa, as shown by Nasir, has been confirmed.

2. The same effect is found, using soil as a medium.

3. There is a definite relationship between the efficiency of the strain used, the incubation period and the concentration of mannitol employed.

4. The feeding action of *Colpidium colpoda* and *Hartmanella hyalina* on *Azotobacter* has been demonstrated.

5. The reason for increased nitrogen fixation appears to be due to the efficiency of *Azotobacter* being maintained for a longer period.

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EXPLANATION OF PLATE XIV.

Fig. 1. Shows the raised growth and normal pigmentation of *Azotobacter* (Flask D) and the gradual disappearance of pigment and growth due to protozoa (Flask H).

Fig. 2. Shows the normal pigmentation and gradual flattening of the growth of an old culture of *Azotobacter* (Flask 6), contrasted with almost complete disappearance of pigment and growth due to the protozoa (Flask 10).

Fig. 3. Shows the stimulation of growth by protozoa in liquid cultures as seen by the cloudiness and scum at the surface. *a*, *b*, *Azotobacter* and *Colpidium colpoda*; *c*, *d*, *Azotobacter* alone.

(*Received June 30th*, 1926.)

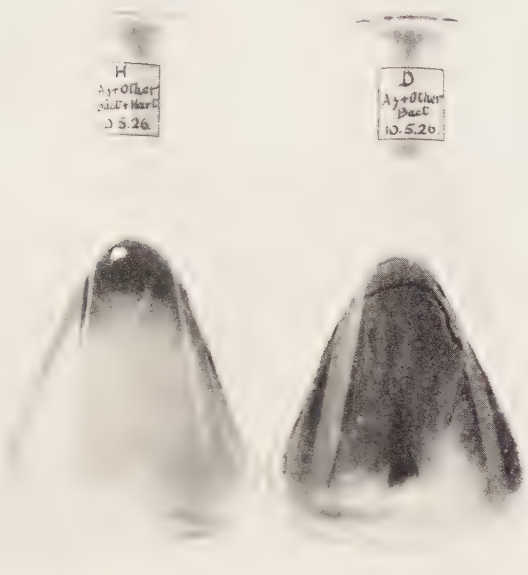

Fig. 1.

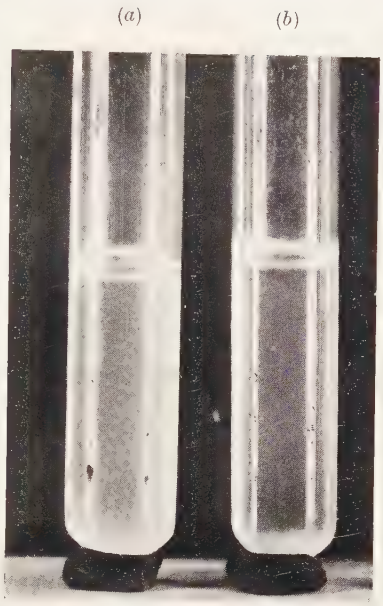

Fig. 3*a*.

Fig. 2.

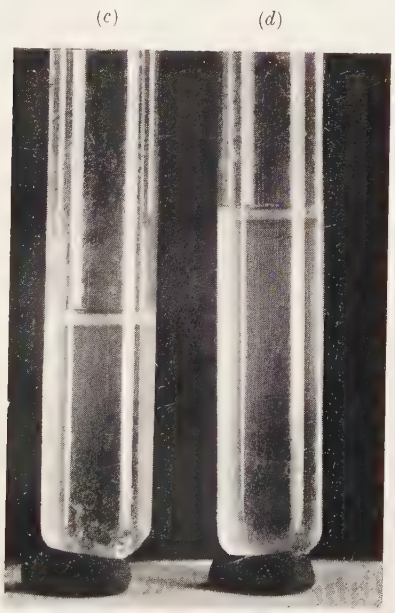

Fig. 3*b*.

YIELD STUDIES IN OATS: THE EFFECT OF THE PRE-TREATMENT OF THE PARENT CROP UPON THE SEED PRODUCED, ITS GERMINATION AND SUBSEQUENT GROWTH

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(With 5 Charts.)

CONTENTS.

I. Introduction.

Few of the agronomic problems of the present would appear to be more complex or fundamental than the analysis of the yield of the cereal crops, involving as it does a large number of inter-related factors operating upon a plastic plant population.

In chronological order of growth the first group of factors to be considered must be the seed characteristics; the term "seed characteristics" is used to designate the effects upon the seed produced by all the climatic, edaphic, and other agronomic factors, constituting the environment of the parent crop.

In the experimental work here reported attention has been directed to these characteristics; the problem was at once limited and more clearly defined by selection of the material—Record Oats being chosen. The method of study pursued was firstly to compare and contrast the germination and growth of samples of the same genetical constitution produced under varying conditions, when grown under conditions

closely approximating to normal field conditions and also when grown under several divergent artificial conditions, attention being paid particularly to the behaviour of the crop as a whole; and secondly, to compare and contrast in a more critical manner the growth of selected seed from two samples of the same bulk, the individual plants more particularly being viewed as the unit.

II. The Effect of Environmental Conditions upon Panicle Organisation.

The effect of environmental conditions on crops of oats grown in 1922 was so marked, more particularly in the size of the grain and in the proportion of grain to straw, that it was decided in 1924 and 1925 to make a further investigation into the question of panicle organisation under sharply contrasted soil conditions and with various times of sowing separated over a wide range.

Plots were grown under field conditions on the trial grounds of the Plant Breeding Station; these were sown in 1924 on February 21st and subsequently for three successive monthly sowings. All the seed was from the same bulk of Record. A plot was also sown on April 22nd on a hill farm at an elevation of 900 feet, the soil being poor and typical of the cultivated land of the district; another plot was grown in the same field but on extra poor soil. The seed rate adopted was nearly double on this plot to what it was on the typical soil, but nevertheless the stand was no thicker. In 1925 plots were sown at the Plant Breeding Station on March 2nd and subsequently every three weeks for three successive sowings. One plot was sown at the hill centre again, this being sown on April 10th, on the more typical soil. Representative samples from all the plots were pulled up by the roots at the time of harvesting and these were later carefully analysed tiller by tiller. The samples showed the tillering to be very low in every case. Quite a number of plants had two or three small side tillers which had only developed some three leaves without showing any signs of sending up a panicle stalk. Consequently all the results have been recorded with the tiller as a unit; the non-panicle-bearing tillers having been ignored; the data presented are average figures for 20 panicle-bearing tillers in each case.

Size of tiller. In both 1924 and 1925 the size of the tiller decreased with later sowing. So also did it decrease with loss of soil fertility, which was at its lowest at the high elevation centre.

The smaller plants, however, were not perfect replicas of the larger on a reduced scale, but varied in panicle organisation. This showed

Table I.

Analysis of Record Oats panicle data from samples grown at a lowland and upland centre.

Centre	Date of sowing	Date of ripening	Average wt. per tiller gm.	Average wt. of grain per panicle excluding steriles gm.	Ratio wt. of grain / wt. of tiller %	Average no. of spikelets per panicle	Average no. of fertile grains per panicle	Average no. of sterile grains per panicle	Average no. of grains per spikelet excluding steriles	Average no. of spikelets per gram of tiller	Average no. of grains per gram of tiller	Grain wt. per 1000 gm.	Husk %
1	2	3	4	5	6	7	8	9	10	11	12	13	14
1924													
Lowland	1. Feb. 22	Sept. 7	4·42	2·21	50	35	61	6·9	1·7	7·9	13·8	36·2	27·6
,,	2. Mar. 21	,, 7	3·36	1·66	49	27	48	4·6	1·8	8·0	14·2	34·6	28·7
,,	3. Apr. 18	,, 11	2·45	1·21	49	21	33	5·3	1·6	8·5	13·5	36·6	26·6
,,	4. May 16	,, 26	1·92	0·83	43	15	24	5·7	1·6	8·0	12·5	34·6	28·0
Hill centre													
Ordinary soil	Apr. 22	,, 27	0·66	0·33	50	7·1	11·5	0·8	1·6	10·8	17·4	28·6	31·2
Very poorest soil	,, 22	,, 27	0·47	0·13	29	4·8	4·8	2·6	1·0	10·2	10·2	27·0	—
1925													
Lowland	1. Mar. 2	Aug. 19	5·16	2·58	50	42	76	3·5	1·8	8·1	14·7	34·0	26·0
,,	2. ,, 23	,, 21	4·20	2·11	50	33	63	1·0	1·9	7·9	15·0	33·5	26·6
,,	3. Apr. 13	,, 25	3·25	1·70	52	28	54	0·1	1·9	8·6	16·6	31·6	28·9
,,	4. May 4	,, 30	2·51	1·32	52	18	37	0·3	2·0	7·4	14·8	35·6	29·1
Hill centre	Apr. 10	,, 28	1·33	0·68	51	16	23	0·0	1·4	12·0	17·3	30·2	26·7
			Total yield cwt. per acre	Grain yield cwt. per acre									
1922													
Lowland	Mar. 27	Sept. 5	65·1	28·0	43	—	—	—	—	—	—	39·4	24·4
Hill centre	Apr. 13	Oct. 7	23·8	4·3	18	—	—	—	—	—	—	24·5	37·2

itself as a reduction in the number of grains per panicle rather than as a reduction in the size of the grain in some cases, whereas in other cases the size of the grain itself was reduced appreciably.

Number of spikelets per panicle. Table I shows that at the Station the number of spikelets per panicle agreed very closely with the weight of the tiller itself, *i.e.* the number of spikelets produced per unit weight (gram) of tiller was fairly constant at about 8 spikelets (see column 11) for the same soil in both years. At the high elevation centre, however, the number (spikelets per gram of tiller) was much higher, especially in 1925.

Number of grains per spikelet. The number of grains developed per spikelet (see column 10) was fairly constant for all the sowings at the Station and also for the ordinary soil conditions at the high elevation. On the extra poor soil, however, the number of grains which developed per spikelet was much lower—due to the high proportion of flowers which remained sterile. The number of grains per spikelet in 1925 was again fairly constant *inter se* for all the sowings at the Station but as a whole higher than in 1924 owing to fewer of the flowers remaining sterile in the latter year (1925). At the high elevation the number of grains per spikelet was considerably reduced, possibly because of the exceptionally large number of spikelets formed in proportion to the size of the plant (see columns 10 and 11). It was particularly noticeable that spikelets with more than 2 grains were very rare in all the samples except that from the last sowing at the Station in 1925.

Number and size of grain. If all the flowers formed were fertilised and developed into grain the number of grains per gram of tiller would have been about equal for all the sowings at the Station in both years. The numbers were, however, very different in the two years owing to the number of flowers which did not produce a kernel, varying according to the weather conditions. The proportion of grain to straw plus chaff, however, remained very constant for all the sowings that were able to ripen properly in both years, so that the average weight of the individual grain produced from the different sowings only varied to a small extent[1]. Some of this small difference was undoubtedly due to the proportion of florets which remained sterile. At the high elevation the number of grains per unit weight (gram) of tiller was much higher, showing that the high proportion of spikelets per unit weight was not fully counter-

[1] Only the average weight of the grain is taken into consideration here. Bowmaker (28) has shown the weight of each individual grain within the panicle to vary within very wide limits, especially in the case of very large panicles.

balanced by the reduced number of grains per spikelet. The tendency towards sterility appeared to be no greater at the high elevation, therefore the number of grains per gram of panicle was high. Nevertheless at the high elevation even with normal growth and ripening conditions the crop only produced the same proportion of grain to the rest of the plant as under the lowland conditions. This limit to the yield of grain per panicle together with the enhanced number of grains resulted in the average size of the grain being smaller even in the most favourable year. Adverse conditions for ripening, following the last of the sowings at the Station in 1924, resulted in seed of a smaller size and poorer quality (lower percentage of kernel) than usual, together with a lower proportion of grain to straw. This phenomenon had been particularly pronounced in 1922, when the season was very late[1]. The Station crop yielded a lower proportion of grain to straw than usual, but the high elevation centre when compared in this respect was even more unsatisfactory, the produce being extraordinarily poor both in its proportion of grain to straw and also in the size and quality of its grain. In 1925 the last sowing at the Station suffered to a certain extent in its early growth from the effect of the drought, but the late rain afterwards favoured it to such an extent that not only did it produce a crop with a high proportion of grains per spikelet—many spikelets having 3 grains—but also produced the largest grains of the season.

Discussion of results.

From the above data it would appear that the character of the soil as judged by both the available supply of plant food and the relative proportions of the essential elements has a marked effect on the size of the tillers produced and also on the organisation of the panicle. Plentiful supplies of nitrogenous manures in the soil are well known to cause the plant in its early stages to produce an abundance of vegetative growth. At the time of the differentiation of the spikelets under these conditions therefore the plant seems to be encouraged to form a large number of spikelets. Each spikelet, presumably, has the potential capacity of producing the maximum number of flowers, which for Record appears to be 4, but at a later stage when differentiation of the flowers is about to take place the precise number which will be developed per spikelet is now governed by the condition of the plant at that time. Under very poor conditions, however, the growth of the plant is generally less vigorous at all stages than under more favourable conditions, and even

[1] See (15), p. 19. (Reference by No. to literature cited.)

with the reduced number of grains per spikelet the plant still may fail to produce seeds of equal size to those on the lowlands even when the conditions for ripening are normal.

Coupled with the effect of the soil on the development of the plant the condition of the weather at different stages of the plant's life has a profound effect, particularly at about the time of ripening. If very cold weather sets in, and especially if accompanied by frosty nights, there is evidence for thinking that the translocation of food material from the rest of the plant to the kernel during the ripening process does not proceed to its maximum and the plant is forced to end off its life activities prematurely, producing seed of a small size and of poor quality (lower proportion of kernel), which of necessity also gives a low proportion of grain to straw. This was shown by the late sowings of 1924 and by the 1922 data. With good soil conditions the plant on the lowlands when subjected to adverse weather conditions generally maintains the size of its grain by reducing the number of spikelets per panicle, and, owing to the season being fairly early on the lowlands as compared with the uplands, the crop generally enjoys fairly good ripening conditions. Owing to the growing season being later to commence in the spring at high elevations, the harvest is naturally later than in the lowlands every year. Late seasons therefore cause abnormal ripening much more frequently in upland districts, so that opportunities of harvesting normally ripened seed occur far more rarely than in the lowlands. This fact, along with the smaller size of the grain even in seasons of normal ripening, makes the high elevation farms generally unsuitable for the production of seed grain.

III. The Seed Samples Employed.

The material chosen came originally from the same bulk of Record Oats, which had been grown on the farm of the Plant Breeding Station in 1923. In 1924, plots were sown on the trial grounds of the Station at four different dates, and the seed samples used for the present experiment were the produce from those four plots, denoted as follows:

Sample	*A.*	Parent crop sown in 1924 on		Feb. 22nd	and ripened on	Sept.	7th
,,	*B.*	,,	,,	March 21st	,,	,,	7th
,,	*C.*	,,	,,	April 18th	,,	,,	11th
,,	*D.*	,,	,,	May 16th	,,	,,	26th

The soil on which these samples were grown in 1924 was a light loam in a fairly fertile condition, having previously been under pasture for several years. Season 1924 on the whole was favourable to the growth

of oats, being sufficiently moist and cool, but the dry and cold March severely retarded and thinned out the February sown crop. The weather conditions at the time of ripening caused the process to be slow and the harvest as such to be late, owing chiefly to the prolonged wet weather and lack of sunshine. Each lot was cut as it ripened and after standing in the stooks on the field for a week, was stored (again in stooks) on a wooden floor in a well-ventilated drying shed. All the lots were threshed on November 5th. The grain was stored in small bags under cool dry conditions. A further sample *E* came from a crop grown at the high altitude trial ground of the Station at Moelglomen, Cardiganshire—an exposed farm at 900 feet above sea level on very poor soil, facing north-west. These oats had been sown on April 22nd, 1924 (using similar seed to that of the *A*, *B*, *C* and *D* lots), and were ripe on September 27th, when they were cut and immediately brought to the drying shed previously referred to, where they remained until threshed on November 5th. It should be noted that the *A*, *B*, *C* and *D* samples were fairly typical of seed harvested from lowland farms when the parent crops are sown at about the dates stated. The *E* sample, however, was plumper than the ordinary seed of the same variety harvested off such poor soil at high elevations and in exposed districts, representing as it did the produce of a year when conditions were exceptionally favourable for the production of good grain at high elevations.

IV. The Seed Samples—Winnowing and Germination Tests, Field Establishment and Yield.

The samples were subjected to drastic winnowing by an exceedingly strong air blast: as is seen from Table II, an exceptionally large proportion of the grain was rejected by this intense seed grading, especially in the case of the poorer samples (*D* and *E*). Chart I shows the germination of the winnowed and unwinnowed samples and indicates the marked superiority of the former in respect of both rate and power of germination.

In order to test the effect upon yield of the seed grading, replicated rows of control seed (non-winnowed) and seed selected by the drastic winnowing method were sown in a large bird-proof cage. Sowing took place on March 17th, 1924, at a rate of 2 seeds per inch at a depth of $\frac{1}{2}$ inch. Germination was very slow, and seedling growth was retarded by the wet, cold weather. The number of plants established varied for the different series, tending to be higher for the winnowed samples, so that the tiller production fluctuated from plant to plant, largely

compensating for irregularities due to defective germination. The rows appeared remarkably even when the plants began to exsert their panicles and no difference in time of ripening was observed between the various samples. No importance can be attached to weight per plant data obtained when the variation from plant to plant is unmistakably large

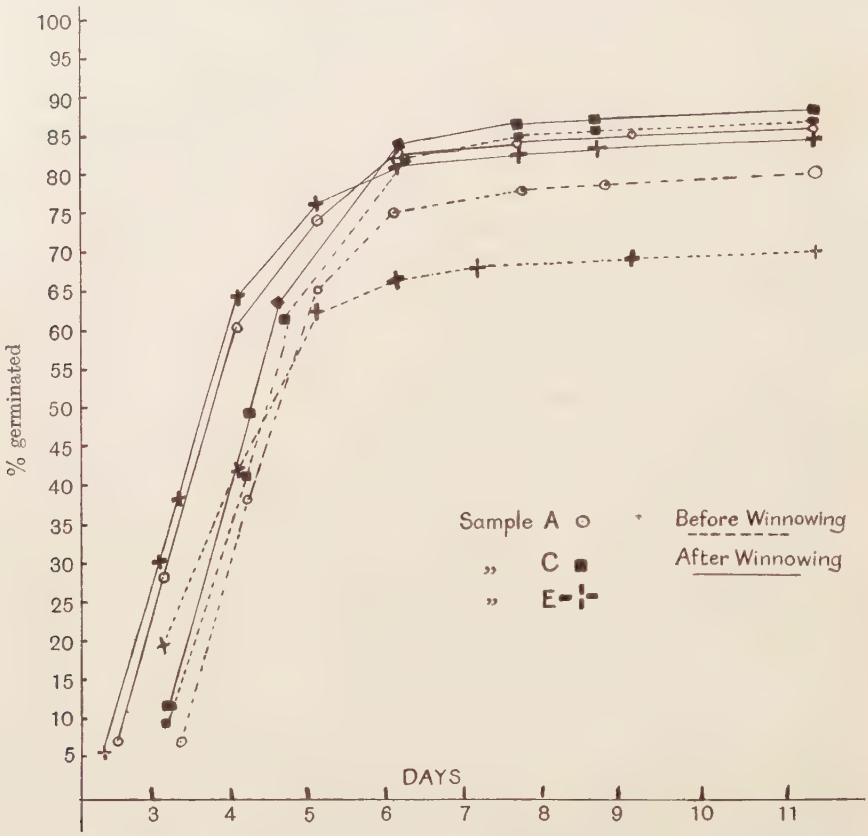

Chart I. The effect of winnowing seed samples upon germination. *C* an ordinary sample from lowlands; *A* a sample of seed from a crop sown very early and grown at the same elevation as *C*; *E* a sample of seed from a crop sown at the same time as *C* but grown at a high altitude.

owing to spacing irregularities, so that it was not found possible to judge the potentiality of the seed samples upon a "per plant basis" in this trial. The yield data are therefore presented per row and it must be noted that although the total number of tillers showed a fairly close agreement per row the number of plants varied very appreciably from row to row.

Table II.

The effect of the hard winnowing on the seed samples and their germination.

	Before winnowing				After winnowing—selected seed					
			Sand germination					Sand germination		
Sample	Wt. per 1000 grain gm.	Moisture content %	Germ. 4th day %	Total germ. %	Selected by winnowing (by wt.) %	Wt. per 1000 grain of selected sample gm.	Moisture content %	Germ. 4th day %	Total germ. %	Rejected seed. Total germ. of rejected sample
A	34·0	15·63	40·0±2·4	85·2±0·75	71·0	35·37	13·45	62·8±1·2	89·4±0·8	80±0·9
B	34·27	16·73	25·7±1·6	85·2±0·9	77·5	35·69	13·25	64·8±1·4	92·0±0·5	76±0·6
C	36·08	16·44	46·0±1·5	91·0±0·8	83·4	36·55	14·00	67·5±1·1	89·1±0·5	89±0·8
D	30·84	15·66	52·6±2·4	87·5±0·8	50·0	36·35	14·01	73·2±1·6	94·0±0·6	88±0·9
E	32·29	15·45	46·3±1·1	75·0±0·9	51·0	37·76	14·05	68·5±1·1	88·3±0·5	77±1·1

Table III.

The effect of winnowing the seed on field establishment and yield.

Notes on parent crop	Seed samples	Percentage established 4 weeks	Percentage established 5 weeks	Av. yield of grain per row gm.	Av. yield of straw per row gm.
Very early sown	*A* Control	24·9	38·8	106·4±5·2	120·2
	A Winnowed	25·2	37·0	96·5±4·4	111·0
Early sown	*B* Control	25·9	35·8	100·3±5·6	107·4
	B Winnowed	26·4	38·5	118·0±5·2	122·9
Normal time of sowing	*C* Control	28·0	39·7	124·7±6·6	134·7
	C Winnowed	26·0	39·3	121·6±6·6	126·5
Late sown	*D* Control	24·1	35·2	85·5±3·3	93·2
	D Winnowed	26·1	38·2	97·6±6·4	97·2
Hill centre	*E* Control	23·4	36·3	116·5±7·1	120·8
	E Winnowed	30·3	44·0	148·3±5·5	148·6
Total yields	Controls	—	—	533·4	576·3
	Winnowed	—	—	582·0	606·2

It is seen from Table III that winnowing the seed samples has increased not only the number of heavy grain and the number of plants established during the first few weeks but also has increased the final yield of grain produced by the hill sample *E*. This mass seed selection has not made the yields of all samples equal; the *A*, *B*, *D* samples (from parent crops sown at unusual dates) have not produced as much grain as the *E* and *C* samples (parent crops sown at normal dates), either when winnowed or sown as harvested. There would therefore appear to be a "seed characteristic" factor other than weight of seed—for the mass selection effected by drastic winnowing made the weight per 1000 of *A*, *B*, *C*, *D* and *E* approximately equal (see Table II). From the early establishment figures it would seem that this seed characteristic manifests itself as a capability to germinate and grow under adverse conditions. It is

therefore seen that the time of sowing the parent crop has influenced the seed immediately resulting somewhat profoundly in relation to its capacity for subsequent crop production. The seed from a parent crop sown too early or too late has not produced such a heavy yield as seed from a crop sown at a more normal time.

V. Growth of Samples under Various Artificial Conditions. Plant Comparisons and Growth Stages.

In order to throw further light upon these "seed characteristics" and in order to obtain data capable of analysis on the "weight per plant" basis a series of trials with spaced seed sown in boxes was carried out. In this trial various simple environmental conditions were artificially induced. It was hoped that a ready method of testing the capabilities of a sample might be found and that behaviour in response to some particular condition would tend to differentiate the samples.

The drastically winnowed samples *A*, *B*, *C*, *D* and *E*, all of practically equal grain weight, were sown in boxes, the seed spaced 2 in. apart and 2 in. from row to row. The treatments to which all the samples were subjected were as follows:

Controls.

Soaked seed. Grain soaked at 22° C. in tap water for 48 hours at the rate of 100 seed (3·5 gm. approx.) per 250 c.c. of water, there being about 3 cm. of water above the grain.

Sand. Normal grain grown in moist, coarse sand, which had been previously well washed.

Dry earth. After sowing these boxes were never watered, so that the seedlings were gradually subjected to drought conditions.

Multiple watering. These boxes received four times as much water as did the control boxes; the soil became thoroughly waterlogged.

Heated grain. The grain was placed in a large oven at 75° C. for 15 minutes just before sowing. The grain acquired the oven temperature in a very short time. This treatment (heating of grain) caused a differential killing effect as is shown by the following figures in Table IV.

The boxes were sown on March 14th, 1925, and immediately placed in a cool greenhouse. The germination of the samples is shown in Table V. It is clearly seen that soaking the grain has hastened germination and that heating the grain has had a marked retarding effect upon the samples (particularly *D*).

It is interesting to note that Stapledon and Adams(25) working with a much lower temperature also observed the differential effect of heating

Table IV.

The effect of heating the seed upon germination.

	Percentage germination, sand tests			
	Control		Heated (for 15 minutes at 75° C.)	
Sample	5th day	Total	5th day	Total
A	90	91	78	91
B	95	97	60	78
C	97	99	71	99
D	92	94	66	83
E	89	92	56	74

on various samples. The work of Groves (12) clearly shows that heated seed resembles very old samples in that not only is the total germination reduced but the rate of germination is slower.

Various growth data accumulated from the several treatments are set out in Tables V, VI and VII.

Table V.

Box germination. Percentage of visible shoots (14 *and* 22 *days*).

	Sample										
	A		*B*		*C*		*D*		*E*		
Treatment	14	22	14	22	14	22	14	22	14	22	Days
Control	57	77	71	81	72	82	82	82	69	77	
Soaked	76	78	76	83	75	75	84	87	61	74	
Multiple water	45	71	46	79	49	82	53	72	49	65	
Dry earth	47	63	42	56	39	41	41	46	32	37	
Sand	62	82	61	84	65	84	71	76	78	83	
Heated grain	24	51	5	32	16	63	2	25	39	66	

Table VI.

Average height in mm. to tip of first leaf of these seedlings (14 *days*).

No. of readings taken per series = 50.

Treatment	*A*	*B*	*C*	*D*	*E*
Control	18·81 ± 0·66	12·12 ± 0·38	13·15 ± 0·37	19·68 ± 0·67	18·23 ± 0·65
Soaked	27·58 ± 0·89	25·69 ± 0·82	30·08 ± 1·10	28·46 ± 1·00	17·44 ± 1·05
Dry earth	16·03 ± 0·64	17·05 ± 0·97	16·22 ± 1·10	19·17 ± 0·86	19·00 ± 1·01

Table VII.

Average height in mm. to tip of first leaf of these seedlings (18 *days*).

Treatment	*A*	*B*	*C*	*D*	*E*
Control	41·92 ± 1·06	38·58 ± 0·73	39·63 ± 0·92	47·92 ± 1·15	48·27 ± 0·92
Soaked	54·72 ± 1·20	55·20 ± 1·40	55·15 ± 2·80	54·46 ± 1·30	42·44 ± 0·89
Multiple water	33·13 ± 1·02	32·92 ± 1·30	34·15 ± 1·15	35·25 ± 1·30	34·91 ± 1·20
Dry earth	38·97 ± 1·18	42·24 ± 1·30	41·42 ± 1·30	46·96 ± 1·80	44·88 ± 1·10
Sand	34·53 ± 0·66	30·63 ± 0·97	36·75 ± 0·88	46·87 ± 0·87	36·52 ± 1·20
Heated grain	30·15 ± 1·20	25·66 ± 1·30	30·20 ± 0·97	26·00*	32·95 ± 1·04

* Average of 25 readings in this case.

It is apparent from an examination of the tables that the initial leaf growth was greatly hastened by the soaking treatment; it is interesting to note, however, that the *E* sample (from the hill centre) has not responded like the others.

A summary of the work on the effect of soaking seed is to be found in Kidd and West's (16) review of the literature. More recently one of us has demonstrated that soaking increases the yield obtained from various Gramineae (28). The multiple water treatment has retarded the first leaf formation, presumably owing to the lower temperature and unfavourable oxygen conditions of the waterlogged soil, all the samples possessing leaves of the same height under such conditions. Atwood (1) working with *Avena fatua* has demonstrated that a lack of oxygen may limit germination. In both sand and dry earth treatments the *D* (late sown) sample stands out from the rest with taller first leaves; nevertheless this sample has suffered from the heating treatment to the greatest extent, only a few plants surviving in the box trials. This high mortality rate is not due to the moisture content of the grain only, for the *C* and *E* samples with a similar percentage moisture (see Table II, p. 543) have not been so reduced in number. The *D* sample did reveal this weakness in the sand germination tests although suffering less than the *E* sample (see Table IV).

On April 30th, that is six weeks after sowing, plants from half of the boxes were removed, measured, grouped in tiller classes, washed, dried at 100° C. and weighed (see Table VIII). It was hoped that it might be possible to find that the different samples when compared would show that the modal plants were at different growth stages. It was at once apparent that a comparison of modal plants was strictly necessary, as already wide variation was to be seen. The data shown are taken from about 75 plants (diseased and otherwise injured plants being rejected) and are shown for the different treatments separately.

From Table VIII it is seen that the various treatments have not as a whole tended to separate the seed samples by their behaviour, the only striking exception to this being the heating treatment which, as previously shown, has proved markedly harmful to the *D* (late sown parent crop) sample.

The data may be examined from another point of view, namely by graphical representation of the mean weight per tiller class (the main axis counted as "1" and the first tiller as "2" (*i.e.* $T_0 + T_1$). From this graphical expression of the data (see Charts II, III and IV) despite the very great differences one must expect even between plants within the

same tiller class[1] it is seen that the various seed samples behave in the same general way on the whole. The *D* sample, however, again seems

Table VIII.

To show various growth data for plants 6 weeks after sowing when subjected to various treatments.

Sample		No. of plants	Average no. of tillers* per plant	Modal no. of leaves fully expanded on shoot	Modal no. of leaves on first shoot	Modal height to uppermost ligule cm.	Average dry weight per 100 plants gm.	Average height to tip of leaf cm.
Control	*A*	73	2·4	3	5	7·1	14·45±0·81	—
	B	80	2·3	3	5	8·0	14·46±0·87	—
	C	79	2·6	3	5	8·2	13·47±0·69	—
	D	72	3·0	3	5	7·0	16·29±0·61	—
	E	70	3·4	3	5	7·1	15·33±0·81	—
Water	*A*	78	2·2	3	4	7·8	11·66±0·56	—
	B	74	2·3	3	5	6·7	13·23±0·55	—
	C	81	2·1	3	5	6·4	11·85±0·84	—
	D	74	3·1	3	5	6·0	11·48±0·98	—
	E	65	3·2	3	5	5·4	12·42±0·67	—
Heated	*A*	58	3·8	3	5	6·7	10·49±0·95	23·85
	B	34	2·7	3	4	5·6	11·85±1·26	22·42
	C	61	2·2	3	5	7·4	10·48±0·46	26·60
	D	27	2·5	3	5	4·5	7·86±0·80	18·10
	E	63	2·9	3	5	7·0	14·03±0·35	26·20
Dry earth	*A*	62	1·5	3	4	4·0	7·27±0·41	17·06
	B	53	1·1	3	4	4·4	6·48±0·33	19·41
	C	50	1·1	3	4	5·9	6·26±0·53	22·80
	D	46	2·0	3	5	3·6	8·49±0·55	16·53
	E	32	2·1	3	5	3·5	7·69±0·90	16·45
Sand	*A*	80	1·0	2	3	3·0	2·06±0·05	10·4
	B	78	1·0	2	3	3·1	2·39±0·06	11·0
	C	80	1·0	2	3	3·0	2·22±0·09	11·0
	D	80	1·0	2	3	2·7	2·42±0·07	9·7
	E	80	1·0	2	3	2·6	2·60±0·06	9·9

* Only living tillers counted.

unique in that it regularly produced plants of a high tiller count actually lighter in weight than those of one tiller less. Engledow and Wadham (7) present similar data showing that several barley plants of a higher tiller class weigh less than those of a lower tiller class. It is interesting to observe that morphological differentiation (tiller production) may proceed independently from physiological growth (dry weight accumulation); this is well shown by the *A*, *B*, *E* graphs at 6 weeks in the dry soil treatment—these series possessing plants of 2, 3 and 4 tillers all approximately of the same dry weight. In the *A* series differentiation

[1] For example a 3-tiller plant may have one main shoot and two well-developed large side tillers, or one main axis and two very small tillers indeed. See (7), *Journ. Agric. Sci.* XIV, pt. 2, 319.

has actually proceeded despite a loss in weight. With an almost complete lack of mineral food, tiller production has not taken place, for all

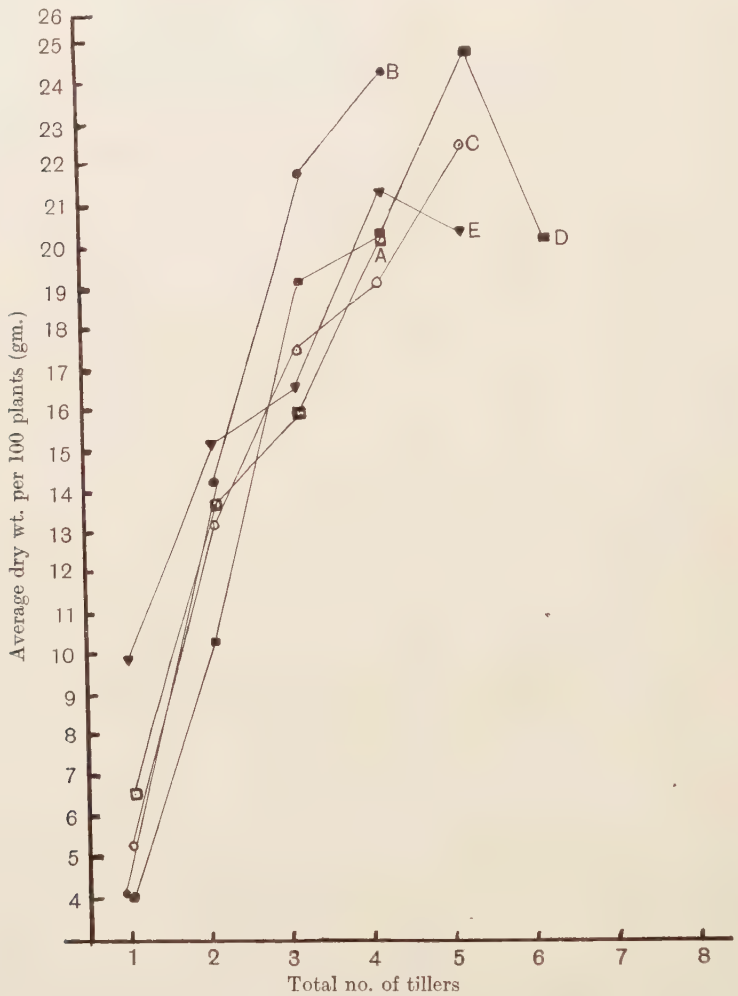

Chart II. Dry weights per tiller class of the five control samples, plants six weeks old.

the plants grown in sand possessed only the main shoot T_0. Leaf differentiation has here not been accompanied by dry weight increase, the final weight of the plants being less than that of the seed. The data obtained from the second harvest at 7 weeks are presented in Table IX.

Table IX.

To show various growth data for plants 7 weeks after sowing when subjected to various treatments.

Sample		No. of plants	Average no. of tillers* per plant	Modal no. of leaves fully expanded on shoot	Modal no. of leaves on first shoot	Modal height to uppermost ligule cm.	Average dry weight per 100 plants gm.	Average height to tip of leaf cm.
Control	*A*	162	2·2	4	5	11·6	22·04±1·8	39·70
	B	164	2·0	3	5	11·8	28·64±1·6	40·92
	C	170	2·1	4	5	10·4	27·14±1·2	36·61
	D	167	2·9	4	5	9·6	34·34±1·8	35·66
	E	155	2·8	4	5	9·6	31·14±1·6	36·67
Dry soil	*A*	56	1·2	3	4	6·2	10·34±0·60	26·06
	B	55	1·2	3	5	5·6	10·38±0·45	22·37
	C	56	1·2	3	4	5·6	10·92±0·29	25·14
	D	56	1·4	3	5	4·2	9·37±0·70	19·50
	E	40	1·4	3	4	5·0	8·51±0·60	22·09
Sand	*A*	88	1·0	2	3	3·1	2·59±0·09	11·02
	B	80	1·0	2	3	2·3	3·12±0·13	10·55
	C	85	1·0	2	3	2·4	2·52±0·07	8·83
	D	77	1·0	2	3	2·1	3·38±0·06	8·75
	E	84	1·0	2	3	2·5	3·10±0·08	8·84

* Only green living tillers counted, dead brown tillers rejected, so that some of the figures in this column are below those in Table VIII. There is evidence of shrinkage in the later formed tillers.

It is apparent from the figures that although all the plants grown in the dry soil have only reached at 7 weeks a growth stage beyond which the controls had progressed at 6 weeks, there is none the less a differential reaction to this subnormal environmental condition by the several seed samples. The *D* sample (late sown parent crop), the control plants of which were remarkably tall and heavy, have proved particularly sensitive to this treatment, the plants being not so tall and not so heavy as those of the *C* sample (parent crop sown at normal date) under the same conditions. In the sand series the plants in no cases developed any side branches but three leaves were differentiated on the main axis. A slight increase in dry weight accompanied this tissue differentiation. There is strong evidence of shrinkage in this series.

VI. Growth of Selected Seed of Equal Weight from an Upland and Lowland Sample of Grain.

From the seed harvested in 1924 from the hill station (*E* sample) and from the sample grown at the Plant Breeding Station (*C* sample) basal grain of the spikelets (*i.e.* "firsts") were selected. The grains were weighed individually and only those selected which were within 10 per

cent. of the average mean weight of the *C* sample ("firsts"); this meant that whereas modal "firsts" were selected from the lowland sample (*C*)

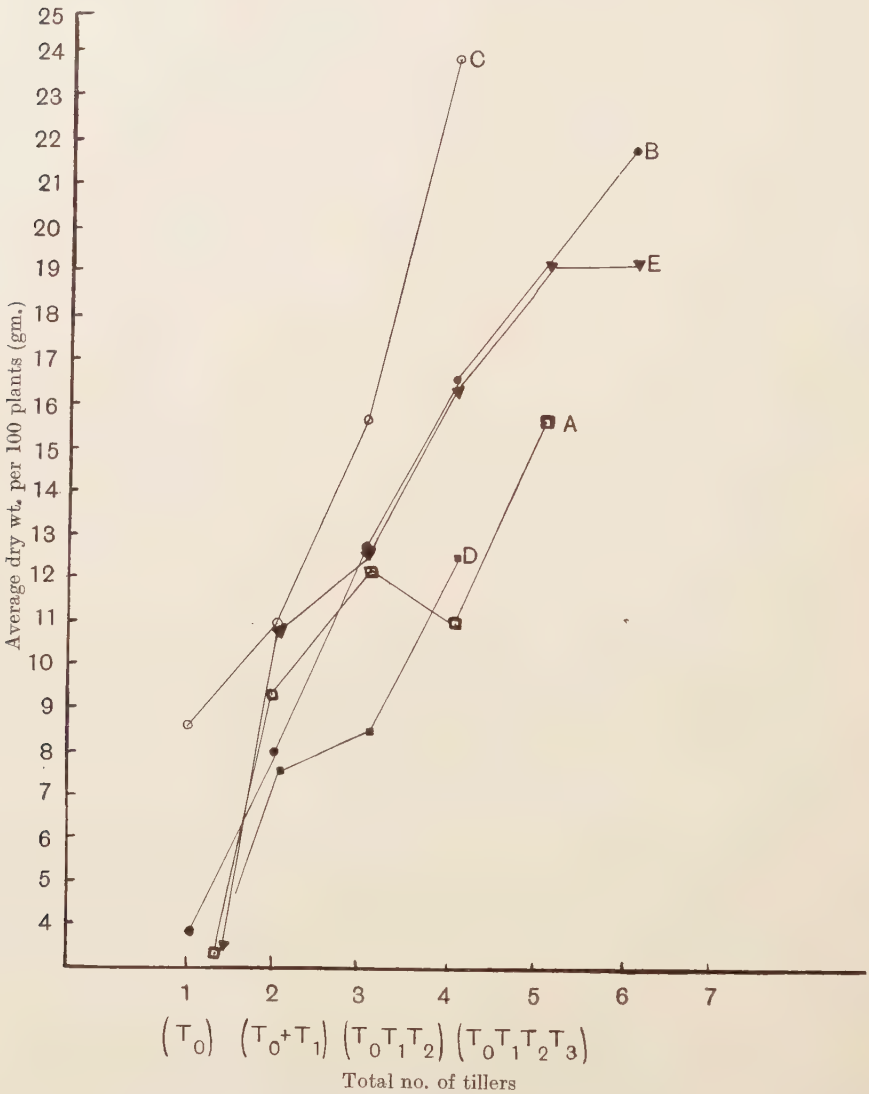

Chart III. Dry weights per tiller class of plants from heated grain, plants six weeks old.

those chosen from the hill sample (*E*) represented heavy "outliers." These seeds were sown on April 17th, 1925, in a large bird-proof cage

in rows. The distance between each seed and each row was 6 in.; under these conditions the factor of competition was almost completely removed and moreover tiller production was allowed full play. Owing to the fact that the establishment was not entirely satisfactory a large number of plants which were not surrounded by a neighbour at 6 in. interval on every side had to be discarded. The data obtained from the

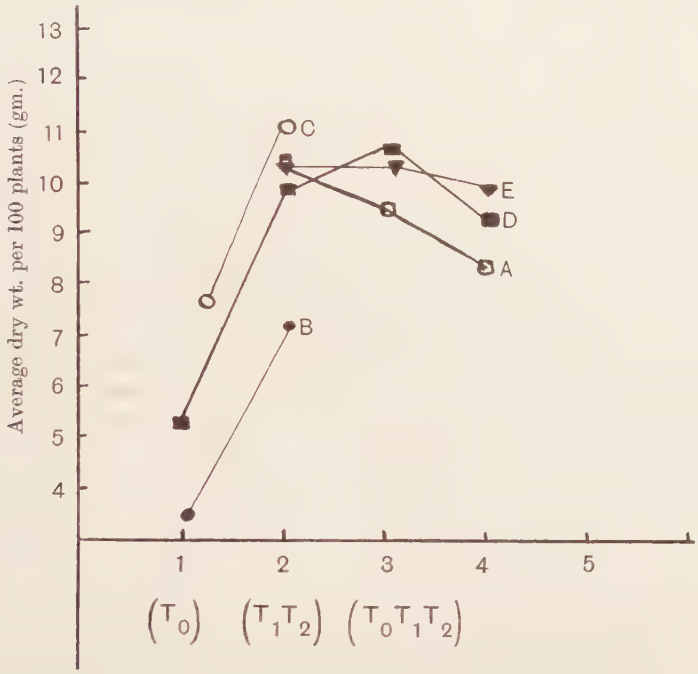

Chart IV. The growth of the samples in dry soil, dry weights per tiller class. (Note that differentiation has proceeded in some cases without dry weight accumulation.)

plants occupying a satisfactory position are summarised in Table X. It was not found possible to obtain data beyond the panicle emergence stage of growth.

From Table X and the graphical representation (see Chart V) of the weight per tiller class, it is seen that the two samples diverge in their behaviour at 11 weeks although somewhat similar at 7 weeks. It is interesting to note, however, that at both stages the lowland (*C*) sample produces a few "outliers" that tiller more profusely than do any plants of the upland (*E*) sample. The average number of tillers, however, is higher in the upland (*E*) sample at 11 weeks. When the average weights

of the tiller classes are compared, whereas at 7 weeks there is little difference, at 11 weeks the modal tiller class of the lowland (*C*) sample weighs more than the modal tiller class of the upland (*E*) sample, although the latter modal class is one of a higher tiller count. The average (mean) weights of all the plants in the two series are equal. The later-formed tillers are not components of yield as they die off without producing a panicle, so that excessive tillering is of no agronomic value.

Table X.

Comparison of the growth of the selected samples.

Age	Data	Lowland (*C*) sample	Upland (*E*) sample
3 weeks.	Percentage established	77·5	57·0
7 weeks.	Av. number of tillers	3·270	3·077
	,, ,, leaves on T_0	5·766	5·846
	,, ,, fully expanded leaves	4·266	4·037
	,, height to tip of longest leaf	24·814 cm.	24·461 cm.
	,, dry weight per plant of the tiller classes:		
	1 tiller —T_0	0·0501 gm.	0·0750 gm.
	2 tillers—T_0, T_1	0·1524 ,,	0·1628 ,,
	3 tillers—T_0, T_1, T_2	0·2924 ,,	0·2765 ,,
	4 tillers—T_0, T_1, T_2, T_3	0·3460 ,,	0·3875 ,,
11 weeks.	*Av. number of tillers	2·654	3·615
	,, height to ligule	53·55 cm.	57·89 cm.
	,, number of panicles visible†	1·077	1·176
	,, height to leaf tip	70·47 cm.	77·67 cm.
	,, dry weight per plant of the tiller classes:		
	1 tiller —T_0	1·500 gm.	1·606 gm.
	2 tillers—T_0, T_1	2·245 ,,	1·658 ,,
	2 tillers—T_0, T_1, T_2	2·040 ,,	1·730 ,,
	4 tillers—T_0, T_1, T_2, T_3	2·435 ,,	2·085 ,,
	Mean weight	2·15±0·043	2·12±0·048

* Dead tillers not counted. † Partially exserted.

In fact it is probable that the plant that produces a few large tillers of a high average and total weight is a preferable unit, from the yield point of view, to one that dissipates its energy in excessive tiller production. It is apparent however that at the panicle emergence stage of growth there were physiological and morphological differences between the plants from the lowland (*C*) sample and those from the upland (*E*) sample of grain. The upland (*E*) sample produced taller and more branched plants coming into panicle slightly earlier, but nevertheless weighing, when compared tiller class with tiller class, less than the plants from the lowland (*C*) samples; moreover the plants of the modal tiller class of lowland (*C*) sample were heavier than those of the modal tiller class of the upland (*E*) sample.

Because the growing season is short (spring oats) a limit is placed

upon the spacing conditions that can be employed in practice to obtain maximum yield per area. Wide spacing results in uneven time of

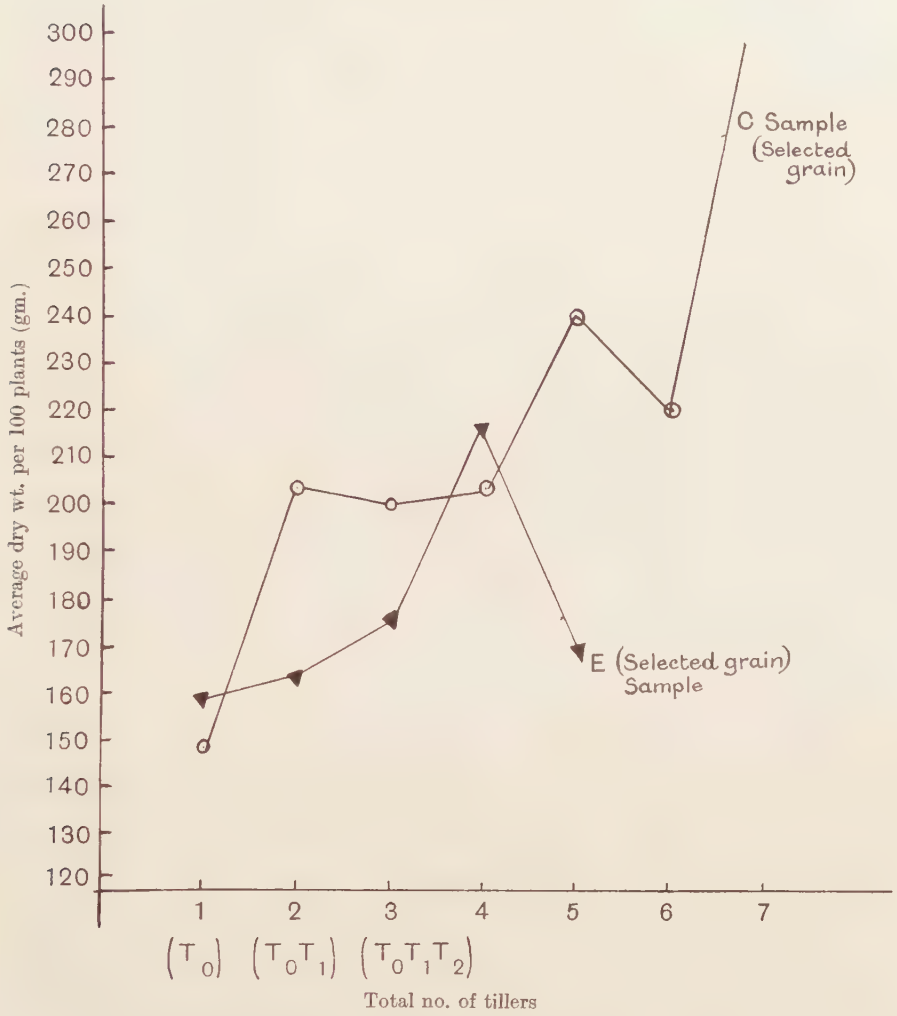

Chart V. Dry weights per tiller class of plants from seed of equal weight selected from *C* and *E* samples. Spaced plants 8? weeks old.

ripening of the tillers. Prescott(22) in his spacing experiments differentiates between the behaviour of maize and wheat from this point of view, whilst an optimum spacing for yield per area exists with maize, wheat by its tillering capacity can be sown throughout a wider range

of spacings. Prescott states that "when only the yield of grain is considered it may happen that small plants are less effective in this respect than larger ones, so that the yield per plant becomes negligible although a certain volume of soil is still available. Under these conditions, well illustrated by maize, an optimum spacing is to be observed." Now if the tiller be reckoned as the unit, and much evidence has been brought forward in support of such a contention, particularly by Engledow and Wadham (7) the above statements of Prescott's might well be applied to oats in this country. The smaller tillers do not make an appreciable contribution towards the yield.

VII. General Discussion.

(*a*) *Weight of seed.* One of the most direct and obvious methods by which the treatment of the parent crop or of the harvested seed predetermines the nature of the growth of the seed produced is through the weight of the seed, for that the weight of seed affects the early growth there can be little or no doubt. Findlay (9), although possibly working with material not constituting a "pure line," found that heavy seeds of oats and barley produced plants which tillered more freely than light seed. Brenchley (4) has shown that heavy seeds give rise to plants with a higher dry weight than do light seeds, and this despite the higher efficiency index of the plants from small seed. The earlier data of this paper show that both time of sowing and environmental conditions affect the mean weight of the seed produced.

(*b*) *Maturity.* As far as possible the grain was harvested ripe. In time the *E* sample from the high altitudes was somewhat late. The state of ripeness has a direct effect upon the germination of the seed. Kondo (17), working with rice, traced out the effect of the state of the seed upon germination and growth. In a previous paper by one of us (15) it has been shown that the state of maturity affects the germination and growth of oat seed. In the case of the trials under review, however, this particular factor was, to all intents and purposes, eliminated. Maturity is very closely related to the meteorological conditions prevailing just before and during harvesting operations. Grieg (11) in 1904 commented upon the beneficent effect of drying in the fields in a comparison between oats harvested in wet weather in Aberdeen and in dry weather in Cambridgeshire.

(*c*) *Panicle organisation—and "time."* The type of spikelet produced is to a certain extent controlled by environmental conditions as was shown by the data discussed in Section II of this paper. The relative

proportions of single-grained spikelets, the size of the grain and particularly the number of spikelets per panicle are amongst the characters of the panicle that seem most readily influenced by seasonal and agronomic conditions. Munro and Beaven (19) working with barley demonstrated that the position of the seed in the inflorescence is correlated with its composition and potentiality, their work would seem to confirm and amplify that of Johannsen. Yamaguchi (27) studied the correlations existing between time of flowering and weight of grain and glumes in the rice inflorescence. Whilst he did not find a high degree of correlation between time of flowering and caryopsis weight, a high positive correlation between time of flowering and glume weight was obtained. There appears, however, to be no doubt that time of flowering is closely correlated with general panicle characteristics. In the samples under consideration the earlier sown (*A* and *B*) and late sown (*D*) samples have not yielded so well in the row trials as the medium sown (*C*) sample, and have been proved to be particularly sensitive to adverse conditions; especially has this been the case with the late sown (*D*) sample.

An optimum date of sowing of the parent crop, such as was found by Prescott, working with maize in Egypt, is therefore shown by our data to exist in the case of spring oats.

(*d*) *Seedling vigour; an index to yield.* The search for an index to yield, the final expression of growth, has been pursued by many workers. Engledow and Wadham working with barley have demonstrated the wide latitude of variation that is possible and probable between plant and plant. The question of tillering investigated by these workers further emphasises the complex nature of the adult cereal plant; with spaced plants and a long growing period it would appear highly improbable that adult growth or yield of the individual graminaceous plants could be foretold by seedling characteristics.

The growth of the individual plant in a crop of spring oats is limited both by the soil space available and by the length of the growth period. Under such conditions the "yield per plant" becomes a factor of less importance and the "number of plants" a highly important factor. Varieties differ in their tillering potentialities and in their capacity for compensating for poor germination and establishment. The very early varieties are particularly susceptible to a period of adverse growing conditions at the commencement of the season, while the later ripening varieties are able to recover and yield fairly well despite the fact that they may be somewhat checked in early growth stages. It has been shown, however, that the largest (presumably the earliest also) tillers

yield best, so that a great number of plants with several well-developed tillers would seem preferable to a smaller number of plants with many tillers, even presupposing that all these tillers would ripen satisfactorily, which is improbable. In any given variety therefore, either early or late, samples of seed which germinate quickly and the seedlings from which establish themselves well, should yield better than samples which germinate less rapidly and produce seedlings that are not so vigorous.

When the literature is surveyed, there would seem to be more than indications that seedling vigour is correlated with yield. Davidson and Stapledon (6) report upon a case of failure of oats in which, despite high total sand germination, the seedlings were not vigorous enough to persist under adverse moisture (field) conditions. Working with maize, Holbert *et alia* (13) conclude that early vigour, as measured by height for example, is closely correlated with final yield. Reed (23), working with an entirely different type of plant—namely *Helianthus*—concludes that plants small or poor at the start generally remain so, and large plants tend to give higher yields at all times. Hughes (14), who also worked with maize, noted that the tallest plants not only gave the most rapid growth increments but also the greatest yield. He further noted that the state of the endosperm also had a predetermining effect, "horny" kernels gave plants that grew larger than those from "starchy" seed. The work of Kyle (18) also bears out the general conclusion that early vigour is correlated with high yielding capacity. Pearl and Surface (20) consider the differences in growth vigour to be due to Mendelian factors distributed at random throughout the mixed population. They also noted that the plants tend to remain in the vigorous or weak category throughout. Ewing (8), searching for correlations between seed-weight characters and growth, found a positive correlation between the weight of the seed on the one hand, and the height of the seedlings, the breadth of leaf, the diameter of stalk and final height of the mature plants on the other hand. The highest figure was obtained between the weight of the seed and the diameter of the stalk. Ewing, however, concluded from his investigations that correlation between fluctuating characters was not of much use to the plant breeder, who above all desires to find a reliable yield index. Pettinger (21) has recently investigated the correlation between yield and early growth of the seedlings in oats; he found a small positive correlation to exist between height of coleoptile and yield, but concludes that this correlation was not high enough to be of biological significance and that length of coleoptile was not a reliable index to yield in oats.

There is, however, further evidence to be found in a slightly different field of work. Seed stimulation as an aid to rapid seedling growth has also been found to affect yield. Gleisburg(10) reports remarkably increased yields of radish from seed soaked in a 15 per cent. solution of magnesium chloride. Kohlrabi seeds were also stimulated to give increased germination and heavier weights of the "tops" of the plants produced. Silbert(24) working with Canadian field peas, soy beans and buckwheat, stimulated the growth throughout the entire growth period of soy beans. Canadian field peas did not respond to the ordinary seed disinfectant treatments but with buckwheat increases in the dry weights of the tops were produced by seed stimulations. From a number of experiments with various economic crops (particularly oats and other Gramineae) the conclusion was reached by one of the writers(26) that the vigour of the growth of the crop was closely correlated with rate of germination. If plant growth be regarded as an exponential series of increments, dry weight being accumulated by a compound interest system, as suggested by Blackman(3), then it would appear perfectly natural that large seedlings should produce large plants. It does not seem unreasonable to suppose that generally (other things being equal) a large plant will yield more grain than a small one. If the rate of increase per unit leaf area be regarded as a better index to the plant's activities, as suggested by Briggs, Kidd and West(5), it would still hold that those seedlings which possessed large first leaves would produce more dry weight—provided that the leaf's activities could commence as soon as those of a smaller leaf and at the same rate.

From all the above literature quoted it would appear that "seedling vigour" is likely to prove an index to yield per area when plants of the same type (or variety) are compared. In the opinion of the writers it would seem that seedling vigour influences yield in spring-sown oats grown under Welsh conditions, governing as it does the all-important factor of establishment. Heating the grain followed by simple germination tests and seedling growth estimates is suggested as one practical method of testing the seed's capabilities. Other methods whereby the seed is subjected to adverse conditions have been briefly outlined.

VIII. SUMMARY.

1. The effect of the parent crop husbandry in the case of oats has been traced upon the seed, its germination and early growth and yield.

2. The beneficial effects of seed grading by weight are again emphasised, but it has been demonstrated that such mass selection does not

cause an improvement of the seed qualities in every respect. More subtle characteristics than weight have a predetermining effect.

3. The relationship between environmental conditions and panicle characters has been discussed.

4. In spring oats grown under Welsh conditions the importance of establishment, which can be roughly gauged by laboratory tests, is emphasised. Heating the seed samples appears to be a suitable method by which further information can be obtained as to the seed's vigour.

5. It seems that an index to yield is likely to be found in this direction. Seed vigour is correlated with yield.

6. Plant to plant variation is so wide between spaced individuals that little indication of the graminaceous plant's capabilities were discernible in seedling behaviour.

It is the very pleasant duty of the writers to express their thanks to Professor R. G. Stapledon for all the help that he has afforded in the work and for his keen interest and very helpful criticisms and suggestions. To Mr Bowmaker thanks are due for his kindness in placing data, laboriously acquired, at the disposal of the writers. The writers are also indebted to Mr E. T. Jones, B.Sc., the cereal geneticist of the Welsh Plant Breeding Station, for his helpful suggestions and criticisms.

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CARBON DIOXIDE IN RELATION TO GLASSHOUSE CROPS.

PART III. THE EFFECT OF ENRICHED ATMOSPHERES ON TOMATOES AND CUCUMBERS.

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(With 2 Text-figures.)

CONTENTS.

Introduction.

Before approaching the main part of this enquiry, it was necessary to determine the amount of carbon dioxide which normally exists in the air of glasshouses, and its variations under working conditions. It was also necessary to devise experimental methods whereby an atmosphere containing any required concentration of this gas could be prepared at will. This work was completed by the end of 1922, and was described in a previous paper (1).

The main problem is concerned with the effect of enriched atmospheres on the marketable crops. Closely allied to this is the question of the production of carbon dioxide on a commercial scale as distinct from the requirements of work which must be considered of a purely experimental nature. In addition to these two important aspects of the problem, the effect of temperature, ventilation and time of day also received consideration.

A partitioned glasshouse was used for the experiments. It was erected in 1923 and consisted of a set of six chambers, the whole being surrounded on three sides by a corridor as represented in Fig. 1. As will be seen from the figure, chambers X 2, X 3 and X 5 were connected with a fan by means of which carbon dioxide, generated in any one of these chambers, could be mixed with the air in that chamber. Suitable dampers were inserted in the pipes so that any one of the three chambers could be treated at will.

As described previously (1) the carbon dioxide was generated from sodium bicarbonate and concentrated sulphuric acid, the generation taking place always inside a chamber. The requisite amount of bicarbonate was mixed with enough water to make a paste in a large earthenware vessel and sufficient sulphuric acid to effect complete decomposition was poured on to it from outside by means of a wide glass tube passing through a wall of the chamber. In this way the gas was quickly generated and the house could remain closed as long as desired. To ensure distribution of the gas the fan was used during the decomposition of the bicarbonate.

To sample the atmosphere of a chamber the operator entered and duplicate samples at any place were taken by displacement. The samples were analysed according to the prescribed manner in the large model of the Haldane apparatus (2). Four analyses were made on each sample and the mean taken.

The tomato plants used in the experiment were treated in accordance with usual manurial practice. All other cultural operations, such as watering, ventilation, trimming and tying, were carried out as in ordinary commercial practice in this district and by experienced workmen.

Experiments in 1923.

The earliest tests showed that in any of the chambers an initial increase of carbon dioxide was soon lost. Furthermore, when any gas generated was mixed with the air by means of the fan, good distribution resulted and the rate of loss followed a smooth curve as already described (1). The following are figures from typical analyses[1]:

Rate of loss.

Time in minutes	Initial (0)	15	30	45	60
Concentration	55·9	50·0	26·4	20·3	13·9
Time in minutes	Initial (0)	20	40	60	80
Concentration	66·0	32·5	19·3	10·7	9·2

[1] The concentrations shown are parts of CO_2 per 10,000 of air.

The following figures are typical of the distribution at two different concentrations:

Distribution.

Samples taken at various points in a house:

(1) 39·0, 39·9, 36·3, 41·2, 41·2

(2) 54·25, 58·3, 54·3, 57·5, 56·5

During the time the work has been in progress checks have been made continuously on the distribution and the rate of loss and it has been found that with concentrations up to and exceeding 1 per cent. of carbon dioxide, the gas is quite well distributed and that there is a regular loss. A point which is examined later, however, is the variation in the initial concentration of the gas. It is found that when the same amount of bicarbonate is decomposed under as near as possible the same conditions on different days the resulting concentration of carbon dioxide is not constant. This point is discussed more fully under the 1925 results (p. 569).

Having determined the approximate rate of loss it was required next to determine the conditions necessary to maintain a high concentration over a period longer than that taken for the complete loss of carbon dioxide generated.

In *X* 2, for instance, it was desired to maintain an average concentration of 90 parts per 10,000 for 1½ to 2 hours daily. This was accomplished by decomposing 7½ lb. of sodium bicarbonate. Theoretically this should give a concentration of about 140 parts per 10,000. Actually the concentration was found to be about 120 parts. In 40 or 50 minutes the concentration fell to about 60 parts. At this stage the decomposition of another 4 lb. of bicarbonate increased the concentration again to 120 parts. After a further 45–50 minutes the concentration fell to about 60 parts. Further quantities of 4 lb. of bicarbonate reproduced the original concentration of 120 parts and a repetition of the process ensured that the concentration lay between 60 and 120 parts, *i.e.* a rough average of 90 parts or 30 times a normal atmospheric concentration of 3 parts.

The same method was used to maintain an average concentration as required in other chambers. Thus in *X* 3 the concentration lay between 90 parts and 30 parts and in *X* 5 between 50 and 10 parts, giving an average of 60 parts and 30 parts respectively. Using this method three of the chambers were treated with the different amounts of carbon dioxide. Chambers *X* 1 and *X* 4 were used as controls. *X* 6 was used to determine the effect of a slow generation of gas at the soil surface.

Previous investigators have studied the effect on plants of the carbon dioxide evolved from soil, and the following experiment was arranged in this connection. Six lengths of 1 in. iron piping perforated with small holes were plugged at one end, and, at the other, connected to a cross piece, the complete arrangement being laid on the soil under the mulching material in *X* 6. The gas was obtained from a cylinder and after passing through a gas meter, was led into the perforated pipes. By this means a continuous generation of gas was obtained at the soil surface. If soil carbon dioxide is a factor in crop production, such an arrangement might be expected to produce a difference in the crop.

Gas was allowed to flow into *X* 6 continuously from June 20th to October 29th at the average rate of 2·45 cubic feet per day. The effect on the composition of the air is shown in Table I, in which figures are quoted at random from a large number of analyses carried out at different times. Sample 1 is taken on the soil surface at two positions, *A* and *B*. Sample 2 is 1 ft. above the soil and sample 3 is from 2 ft. above the soil.

Table I.

Showing the effect of liberating CO_2 from pipes laid under the mulch of stable manure on the amount present in the air.

1		2		3	
A	*B*	*A*	*B*	*A*	*B*
4·2	4·3	4·8	4·7	3·4	3·2
3·3	3·1	2·4	2·4	2·4	2·5
3·8	3·7	4·0	4·0	5·1	4·4
2·6	4·2	3·0	2·9	4·0	3·9
2·9	2·8	3·5	3·6	3·5	3·7

The figures in Table II show how the extra carbon dioxide supplied in *X* 6 affects the concentration at the soil surface compared with other houses (*X* 4 and *X* 5) where the only source of carbon dioxide at the time was that naturally formed. The samples were all taken on the soil surface at two points *A* and *B* which correspond for the three chambers.

Table II.

Showing the effect of soil CO_2 on the amount present in the air.

X 6		*X* 5 (control)		*X* 4 (control)	
A	*B*	*A*	*B*	*A*	*B*
3·4	4·9	1·9	1·9	2·2	2·2
2·3	2·1	3·2	1·7	1·9	1·8
4·7	4·8	3·4	2·1	2·3	2·2
2·6	2·4	2·7	2·5	2·4	2·6
2·0	1·9	1·2	1·1	0·9	0·9

Tables I and II suggest that the slow generation of the carbon dioxide at the soil surface does not have any constant effect on the atmosphere immediately over the soil.

Following these preliminaries, chambers were arranged in which normal atmospheres were compared with those containing 10 times normal, 20 times normal and 30 times normal carbon dioxide. Owing to difficulty with the heating of the chambers in the first year of construction, much emphasis could not be laid on the results, but there were indications that the tomato crop can be increased by artificially enriching the air of glasshouses with CO_2.

A point of interest which arose at the end of the growing period of 1923 concerns the state of the roots of the plants in the different chambers. Since this was the first season in which tomatoes had been grown in the particular soil it was reasonable to suppose that the roots would be reasonably free from disease. In accordance with the usual practice at this station, the root of each plant in the chambers was examined for disease at the end of the season. The organism chiefly concerned was *Colletotrichum atramentarium.* The results detailed in Table III suggest that the large amounts of carbon dioxide render the roots susceptible to infection by this organism.

Table III.

Chamber	Treatment	Percentage diseased roots
X 1	Control	13
X 2	90 parts CO_2	68
X 3	60 ,,	26
X 4	Control	14
X 5	30 parts CO_2	32
X 6	Soil CO_2	50

Experiments in 1924.

By the end of 1923 the temperature control had been much improved so that it was possible to keep the temperatures uniform throughout the chambers. Temperature records were taken and it was found that under the conditions of the experiment the temperatures were approximately the same in all chambers.

It was decided that the following points should be examined:

(1) Effect of treatment at different stages of growth.
(2) ,, ,, on germination.
(3) ,, ,, on the monthly pickings.
(4) ,, irregular treatment.
(5) ,, treatment on the incidence of root disease.

Effect of treatment at different stages of growth of plants.

Several lots of plants were raised under different conditions, and grown to maturity, and it was necessary to divide *X* 5 and *X* 2 into two plots each, *i.e.* *X* 5 *a*, *X* 5 *b* and *X* 2 *a*, *X* 2 *b* respectively. The plots were then arranged as follows:

X 1. Control.

X 2 *a*. Plants treated for 2 hr. 40 min. with 90 parts daily from time of sowing till planted out, then with 60 parts till end of the growing season.

X 2 *b*. Plants treated for 2 hr. 40 min. daily with 60 parts *after* planting out.

X 3. Irregular treatment.

X 4. Control.

X 5 *a*. Plants treated for 2 hr. 40 min. daily with 90 parts from time of sowing till planted out only.

X 5 *b*. Plants treated for 2 hr. 40 min. daily with 90 parts from time of sowing till ready for potting only.

X 6. As in 1923, *i.e.* plants exposed to a continuous generation of gas at the soil surface.

In each chamber were 56 plants, the half plots in *X* 2 and *X* 5 having 28 plants each. The variety used was "Ailsa Craig."

The dates of the cultural operations were as follows:

Seeds sown, January 1st, 1924.
Plants potted, January 28th, 1924.

The effects of the treatment on the crops which are given in detail in Tables IX and XVI are summarised in Table IV.

Table IV.

Chamber	Treatment	Lb. per plant	Relative
X 1	Control	8·59	—
X 2 *a*	90 parts in boxes and pots, 60 parts after planting	9·64	119
X 2 *b*	60 parts after planting	10·14	125
X 3	Irregular treatment	8·91	110
X 4	Control	7·59	—
X 5 *a*	90 parts in boxes and pots	8·54	106
X 5 *b*	90 parts in boxes	9·21	114
X 6	CO_2 from soil surface	8·41	104
	Average of controls	8·09	100

The figures for *X a* and *X* 2 *b* suggest that no particular benefit is likely to result from treatment applied before the plants are in the

houses. Actually, during their life history the plants in *X* 2 *a* received more carbon dioxide than those in *X* 2 *b*, and those in *X* 5 *a* received more than those in *X* 5 *b*, yet the crops in the *b* plots in both cases are higher than those in the *a* plots. This, with the concentration of gas used, is hardly likely to be due to the treatment. It will be seen from Fig. 1 that *X* 2 *b* and *X* 5 *b* are both on the corridor side of the chambers. The other sides, namely *X* 2 *a* and *X* 5 *a* are well sheltered since another block of houses is only 4 ft. away on that side. Thus it is not likely to be a temperature factor but rather the differences in the plots are due to their being on that side of each chamber. The main point is that an increase of 19 per cent. in marketable produce has resulted from the treatment given in *X* 2 *a* and an increase of 25 per cent. in *X* 2 *b*.

The increase in *X* 6 is not considered sufficiently high, in comparison with *X* 5 and *X* 2, to warrant further experiment. The increase in crop is very small, the method is somewhat costly and the question of increasing the carbon dioxide supplied in this way is not easy of solution.

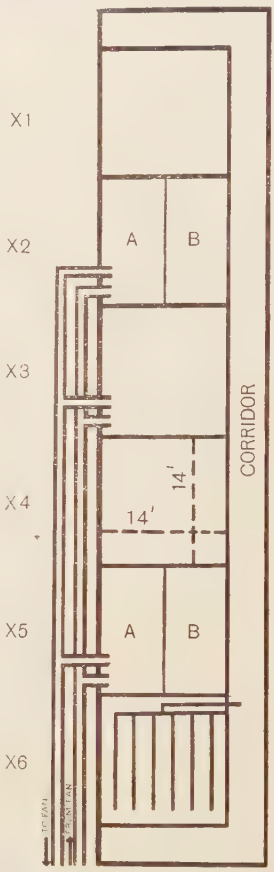

Fig. 1. Showing the arrangement of the experimental chambers.

Effect on rate of germination.

Observations were made on 500 seeds sown in standard seed boxes in the chamber in *X* 2 where there was an average concentration of 90 parts per 10,000 of carbon dioxide from 9.30 a.m. for 2 hr. 40 min. daily. Control boxes were kept in *X* 1. Temperature and other factors were as far as possible the same in both chambers. Analyses of the air immediately over each box were carried out practically every day. The results of germination are shown in the graphs in Fig. 2.

When the seedlings were ready for potting there appeared to be very little difference between those treated and the controls. As will be seen from the graphs the treatment had little effect and it would appear, therefore, that no advantage is gained from germinating tomato seeds in an enriched atmosphere.

Effect on monthly pickings.

The total pickings per month, in pounds per plant, for the different chambers are shown in Table V.

Table V.

	May	June	July	Aug.	Sept.	Oct.	Total
Control	0·82	2·16	1·51	1·72	1·22	0·65	8·09
2 *a*	0·83	3·02	1·75	1·82	1·65	0·57	9·64
2 *b*	1·24	3·05	1·72	1·79	1·73	0·61	10·14
3	1·09	2·26	1·66	1·96	1·40	0·60	8·91
5 *a*	0·71	2·63	1·57	1·62	1·39	0·62	8·54
5 *b*	0·84	2·52	1·65	1·53	1·81	0·86	9·21
6	0·88	1·69	1·56	2·10	1·72	0·46	8·41

Examination of the figures shows that except in the case of *X* 6 and *X* 5 *a* in May, all the pickings in the treated chambers are slightly

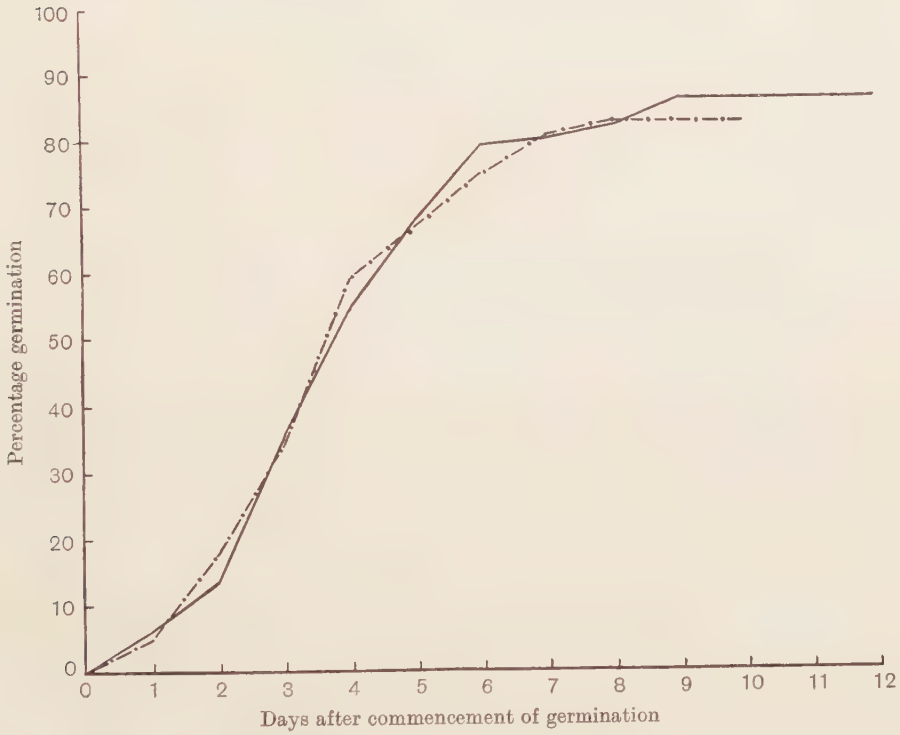

Fig. 2.

higher than those in the controls. It is evident that where the gas is mixed with the air at the concentrations named no effect in the crop is found at the end of the season, so that to benefit by the treatment plants

need not be treated later than the beginning of September. In *X* 6 a different state of affairs exists. Here no appreciable increase occurs till August and is only in evidence during this month and September.

Effect of irregular treatment.

It was originally intended to subject the plants in *X* 3 to high concentrations of the order of 3 to 4 per cent. This was to have been supplied by a large-scale apparatus but unfortunately such concentrations were never obtained and it was only possible to pump gas into the chamber when the apparatus was available. Consequently intermittent doses of the gas were pumped in. As a rule the resulting concentration was small —from 10 to 25 parts CO_2. From Table IV it is clear that the plants do benefit from this but the crop increase is not as great as when the treatment is regular and the concentration of gas is higher, as in *X* 2.

Experiments in 1925.

For the actual trials only four chambers were used. Two were devoted to tomatoes and two to cucumbers, the chambers being arranged as follows:

X 1. Fourteen cucumber plants—variety, Butcher's Disease Resister—control, no carbon dioxide supplied.

X 2. Plants as in *X* 1—treated with an average concentration of 90 parts CO_2 from 9.30 a.m. to 10.30 a.m. daily after planting.

X 3. Fifty-six tomato plants—variety, Tuckswood—treated with 60 parts CO_2 from 8.30 a.m. to 9.30 a.m. daily after planting.

X 4. Plants as in *X* 3—control, no carbon dioxide supplied.

All normal cultural conditions, such as temperature, ventilation and manurial treatment, were kept comparable in the various chambers. Temperature records were taken throughout the season and the average temperature was found to be similar in the respective pairs of treated and untreated chambers.

The dates of the various operations were as follows:

Tomatoes planted March 3rd, 1925.
Cucumbers planted March 10th, 1925.
Carbon dioxide treatment commenced March 24th, 1925.
„ „ ceased September 10th, 1925.

The results are summarised in Table VI. For tomatoes the detailed pickings are given in Tables XVIII and XIX of the Appendix.

Table VI.

Chamber		Lb. per plant	Relative
X 1	Cucumbers—control	38·45	100
X 2	,, —90 parts CO_2 after planting	44·49	116
X 3	Tomatoes —60 parts CO_2 after planting	7·28	125
X 4	,, —control	5·81	100

These results confirm those obtained in 1924 and show that the treatment is responsible for an increase of 25 per cent. in a tomato crop. In the case of cucumbers the increase is only 16 per cent. During the season little difference was evident in the appearance of the plants till the end of the season. Then it was noticed that the treated tomato plants were somewhat fresher in appearance and were certainly taller than the untreated plants. The treated cucumber plants lived for some time longer than the control plants. Table VII gives the monthly tomato pickings in *X* 3 and *X* 4 in pounds per plant.

Table VII.

	May	June	July	Aug.	Sept.	Oct.	Total
X 3	0·81	2·69	0·92	1·30	0·93	0·63	7·28
X 4	0·97	1·44	0·90	1·37	0·53	0·60	5·81

It is clear that the increase in crop is confined to the months of June and September. Having regard to the enhanced value of May and June pickings, commercial interest centres round this increase. Were it possible to produce such an early increase in crop regularly the growth of two successive tomato crops in one season under these conditions might be very profitable. Treatment of the second crop would give a big increase in September and it might be possible to keep a second crop till the end of October or even later, when prices are on the up-grade. These possibilities are being examined in 1926.

Effect of Treatment on Quality of Fruit.

Examination of the various tables in the Appendix will show that the quality of the fruit, and consequently its market value, is unaffected by the treatment.

The Loss of Carbon Dioxide.

In any process for supplying carbon dioxide on a big scale in glasshouses a very important point is the relation between the absolute amount of gas generated or supplied and the amount actually found present on analysis. It has already been pointed out that whenever an

accumulation of carbon dioxide occurs—whether naturally, as at night in a glasshouse, or artificially, as in these experiments—a loss always occurs in daylight. This loss does not appear to be due, in its entirety at all events, to assimilation. It appears probable that most of the loss is due to leakage. It was considered of interest to determine whether such loss could be associated with any variable factor such as temperature. It did appear during the course of the work that the rate of loss was greater in bright sunny weather than in cloudy or rainy weather. It was considered that the main factor here was temperature. So it was decided to examine the effect of temperature on this loss. The results in Table VIII were obtained in chambers *X* 2, *X* 3 and *X* 5, and unless stated otherwise the weather was bright and sunny. It will be seen that the initial loss cannot be associated with temperature.

Table VIII.

Chamber	Temp. ° F.	Initial concentration	Concentration after 1 hour	
X 2. Theoretically, 190 parts	65	158·6	109·7	Raining
	67	175	100·2	—
	68	168·1	77·9	—
	70	186·4	140·3	Raining
	72	154·7	95	—
	76	144·8	74·6	—
	82·5	160·5	92·5	—
X 3. Theoretically, 114 parts	60	100·1	71·4	Raining
	63	108·3	50·3	—
	64	110·6	83·8	Raining
	68	108·2	87·3	,,
	70	95·6	32·2	—
	67	102·8	32·7	—
	71	111·3	63	—
	70	81·1	22·6	—
X 5. Theoretically, 114 parts	67	103	35·9	—
	68	117·4	76·2	—
	75	107·9	42·5	—
	83	110·5	29·5	—
	83·5	99·5	27·6	—
	85	94	—	—

That temperature is not responsible for the whole loss is well shown by the figures in Table VIII for *X* 3. In this case, of a theoretical 114 parts only 110 parts are found at most and that at 64° F. and 71° F., two appreciably different temperatures. On the other hand, at a temperature of 70° F. on one occasion a concentration of 95·6 parts was found but on another occasion only 81·1 parts were present. The other figures given bear this out. It must be concluded, therefore, that the amount of the carbon dioxide lost is not associated with the temperature in a house.

Effect of ventilation on the concentration.

At certain times of the season it is impossible to keep tomato houses shut down. On hot days, the shutting of ventilators, for an hour only, produces temperature increases which have undesirable effects on the plants. It was essential, therefore, to determine the effect of ventilation on the initial concentration of gas. Analyses showed that when the ventilators were open during the decomposition the loss was much greater than normally. This, of course, is to be expected. Typical results are quoted for *X* 5:

Theoretical concentration 114 parts.
Usual concentration found 100–105 parts.
Concentration with 2 top ventilators open 61·6 parts.
,, ,, 4 ,, ,, 35·5 ,,
,, ,, 4 top and 2 side ventilators open 14·8 parts.

Thus the amount of loss increases with increase in ventilation. Consequently the generation of gas must be carried out with the house closed down.

Experiments with Commercial Apparatus.

Since carbon dioxide was first suggested some years ago as a means of increasing crop production several attempts have been made to use the gas on a large scale. In the earliest experiments the waste gases from high temperature furnaces were used as a source of the gas. The waste gases, after suitable purification, were pumped to the desired area. Subsequent work has tended to evolve self-contained apparatus to produce, and, where necessary, purify the gas. Experiments with this type have been made in various parts of the world, including this country and the Channel Islands, but the results were not promising. More recently, improved models have been devised and the inventor of one of these undertook to supervise the installation of his apparatus at the station in time for the 1923 season. In this particular apparatus carbon dioxide is made by burning coal, or preferably coke. The gas is washed with water and then passed into a solution of potassium carbonate. The mixture of potassium carbonate and bicarbonate is then heated and the resulting carbon dioxide is used as desired. The residual carbonate is used again for absorption and the process repeats itself so that there is a continuous cycle.

The crop from the plants treated with gas made in this apparatus was no better than that from plants in the adjoining house which

received the same manurial treatment but no carbon dioxide, so that the gas supplied was insufficient to produce the expected results.

At the end of 1924 the apparatus was removed and replaced by a larger model with some modifications. The principle was exactly the same as in the earlier model. A house of 23,600 cubic feet capacity was used for the tests and gas pumped in every day. No effect was produced on the crop and it is probable that the concentrations of the gas produced in this way are too low to have a favourable effect on the plants.

An attempt was made to determine whether a quick generation of gas would give a high concentration in a house of this size. For this purpose 74 lb. of sodium bicarbonate in a large earthenware vessel were decomposed with sulphuric acid and the gas pumped into the house. An average of eight samples of the air in the house, taken 25 minutes after the commencement of the decomposition, showed that 59 parts were present. A sample from the supply pipe showed 422 parts. An average of four samples of air taken in the house 45 minutes after complete decomposition showed the presence of 29 parts carbon dioxide. It appears, therefore, that even in a big house, a quick supply of gas will produce a concentration sufficiently high for the purpose aimed at.

Providing apparatus can be devised for producing the large amount of gas required there is no reason to suppose that it would not be possible to bring about a crop increase comparable to that obtained in the small chambers.

Summary.

(1) The rate of germination of tomato seeds is practically unaffected when the seed boxes are exposed to atmospheres enriched with carbon dioxide.

(2) Increases of over 20 per cent. in marketable tomato crops result when the plants are subjected to atmospheres containing 0·6 per cent. of CO_2 for 1 or 2 hours daily.

(3) Cucumbers heated with atmospheres containing 0·9 per cent. CO_2 for 1 hour daily showed an increase in crop of 16 per cent.

(4) In the case of the 1925 tomato crop, and to a less degree of the 1924 crop, a considerable part of the increased picking is manifested in June, when market prices are high.

(5) In its effect on the crop carbon dioxide generated slowly at the soil surface is not as beneficial as the gas when generated quickly and mixed with the air surrounding the plants.

(6) Plants grown in houses which have been used for carbon dioxide experiments show an increased susceptibility to infection by *Colletotrichum atramentarium*.

(7) When carbon dioxide is generated quickly in a house an appreciable loss of this gas always occurs. This loss appears to be independent of temperature and varies directly with the amount of ventilation.

(8) Experiments with apparatus devised for the use of carbon dioxide on a commercial scale were unsuccessful owing to the inadequacy of the apparatus.

The authors are indebted to Dr W. F. Bewley, whose advice and criticism have been invaluable during the progress of the work. Grateful acknowledgments are also due to Sir John Russell and Professor V. H. Blackman for valuable suggestions made from time to time.

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Appendix.

Tables IX–XVI show details of tomato pickings for 1924, in pounds per plant.

Tables XVII and XVIII show details of tomato pickings for 1925, in pounds per plant.

Explanation of grading system:

A. The best quality of fruit in size, shape and colour.
B. Fruit of good shape and colour but smaller than Grade A.
C. Fruit of good colour but larger and more irregular in shape than Grade A.
D. Small misshapen fruits about the size of cherries.
E. Blotchy fruits which ripen irregularly.
F. Fruits showing Blossom End Rot.
G. Fruits showing lesions of Stripe disease.

Table IX.

X 1

	Lb. per plant							Percentage
Grade	May	June	July	Aug.	Sept.	Oct.	Total	of total
A	0·70	1·45	0·97	1·41	0·64	0·40	5·57	64·85
B	0·04	0·22	0·23	0·33	0·28	0·22	1·32	15·37
C	0·04	0·69	0·31	0·19	0·07	0·04	1·34	15·61
D	—	—	0·02	0·04	0·08	0·06	0·20	2·32
E	0·01	0·08	0·01	—	0·01	—	0·11	1·28
F	0·01	0·01	—	—	—	—	0·02	0·23
G	—	0·03	—	—	—	—	0·03	0·34
Total	0·80	2·48	1·54	1·97	1·08	0·72	8·59	100·00

Table X.

X 2 *a*	Lb. per plant							Percentage
Grade	May	June	July	Aug.	Sept.	Oct.	Total	of total
A	0·79	1·86	1·27	1·40	1·01	0·27	6·60	68·47
B	0·03	0·38	0·26	0·28	0·53	0·21	1·69	17·53
C	0·01	0·63	0·21	0·12	0·04	0·08	1·09	11·31
D	—	—	—	0·02	0·07	0·01	0·10	1·03
E	—	0·15	0·01	—	—	—	0·16	1·66
F	—	—	—	—	—	—	—	—
G	—	—	—	—	—	—	—	—
Total	0·83	3·02	1·75	1·82	1·65	0·57	9·64	100·00

Table XI.

X 2 *b*	Lb. per plant							Percentage
Grade	May	June	July	Aug.	Sept.	Oct.	Total	of total
A	1·06	1·89	1·21	1·16	1·19	0·35	6·86	67·64
B	0·05	0·37	0·27	0·34	0·38	0·22	1·63	16·08
C	0·13	0·70	0·22	0·27	0·07	0·01	1·40	13·81
D	—	—	—	0·02	0·09	0·03	0·14	1·38
E	—	0·09	—	—	—	—	0·09	0·89
F	—	—	—	—	—	—	—	—
G	—	—	0·02	—	—	—	0·02	0·20
Total	1·24	3·05	1·72	1·79	1·73	0·61	10·14	100·00

Table XII.

X 3	Lb. per plant							Percentage
Grade	May	June	July	Aug.	Sept.	Oct.	Total	of total
A	0·90	1·37	1·02	1·32	0·85	0·33	5·79	64·98
B	0·10	0·29	0·21	0·42	0·32	0·18	1·52	17·06
C	0·07	0·43	0·28	0·21	0·09	0·03	1·11	12·46
D	0·01	—	—	0·01	0·14	0·06	0·22	2·47
E	—	0·13	0·09	—	—	—	0·22	2·47
F	0·01	0·01	—	—	—	—	0·02	0·22
G	—	0·03	—	—	—	—	0·03	0·34
Total	1·09	2·26	1·60	1·96	1·40	0·60	8·91	100·00

Table XIII.

X 4	Lb. per plant							Percentage
Grade	May	June	July	Aug.	Sept.	Oct.	Total	of total
A	0·67	1·03	1·00	1·18	0·93	0·32	5·13	67·58
B	0·13	0·28	0·26	0·17	0·25	0·15	1·24	16·34
C	0·03	0·26	0·16	0·09	0·08	0·05	0·67	8·83
D	—	—	—	0·03	0·10	0·05	0·18	2·37
E	—	0·23	0·05	—	—	—	0·28	3·69
F	0·01	0·03	—	—	—	—	0·04	0·53
G	—	0·04	0·01	—	—	—	0·05	0·66
Total	0·84	1·87	1·48	1·47	1·36	0·57	7·59	100·00

Table XIV.

X 5 a

Grade	Lb. per plant							Percentage of total
	May	June	July	Aug.	Sept.	Oct.	Total	
A	0·63	1·80	0·92	1·22	0·96	0·22	5·75	67·33
B	0·06	0·39	0·24	0·20	0·26	0·32	1·47	17·22
C	0·02	0·36	0·35	0·16	0·09	0·07	1·05	12·29
D	—	—	—	0·04	0·08	0·01	0·13	1·52
E	—	0·05	0·06	—	—	—	0·11	1·29
F	—	0·03	—	—	—	—	0·03	0·35
G	—	—	—	—	—	—	—	-
Total	0·71	2·63	1·57	1·62	1·39	0·62	8·54	100·00

Table XV.

X 5 b

Grade	Lb. per plant							Percentage of total
	May	June	July	Aug.	Sept.	Oct.	Total	
A	0·71	1·55	1·09	1·20	1·33	0·44	6·32	68·62
B	0·11	0·35	0·35	0·25	0·31	0·30	1·67	18·13
C	0·02	0·43	0·19	0·08	0·12	0·03	0·87	9·45
D	—	—	—	—	0·05	0·09	0·14	1·52
E	—	0·17	0·02	—	—	—	0·19	2·06
F	—	0·01	—	—	—	—	0·01	0·11
G	—	0·01	—	—	—	—	0·01	0·11
Total	0·84	2·52	1·65	1·53	1·81	0·86	9·21	100·00

Table XVI.

X 6

Grade	Lb. per plant							Percentage of total
	May	June	July	Aug.	Sept.	Oct.	Total	
A	0·72	0·91	1·20	1·63	1·09	0·18	5·73	68·14
B	0·13	0·24	0·20	0·24	0·42	0·23	1·46	17·36
C	0·02	0·44	0·12	0·19	0·17	0·02	0·96	11·41
D	—	—	0·01	0·04	0·04	0·03	0·12	1·43
E	—	0·03	0·03	—	—	—	0·06	0·71
F	0·01	0·01	—	—	—	—	0·02	0·24
G	—	0·06	—	—	—	—	0·06	0·71
Total	0·88	1·69	1·56	2·10	1·72	0·46	8·41	100·00

Table XVII.

X 3

Grade	Lb. per plant							Percentage of total
	May	June	July	Aug.	Sept.	Oct.	Total	
A	0·54	1·55	0·40	0·88	0·39	0·40	4·16	57·15
B	0·13	0·23	0·23	0·11	0·13	0·19	1·02	14·02
C	0·12	0·86	0·28	0·25	0·34	0·04	1·89	26·01
D	—	—	—	0·02	0·07	—	0·09	1·23
E	0·01	0·05	0·01	0·03	—	—	0·10	1·33
F	0·01	—	—	—	—	—	0·01	0·13
G	—	—	—	0·01	—	—	0·01	0·13
Total	0·81	2·69	0·92	1·30	0·93	0·63	7·28	100·00

Table XVIII.

X 4	Lb. per plant							Percentage of total
Grade	May	June	July	Aug.	Sept.	Oct.	Total	
A	0·64	0·75	0·46	0·99	0·21	0·30	3·35	57·65
B	0·17	0·21	0·17	0·11	0·11	0·22	0·99	17·03
C	0·10	0·44	0·24	0·26	0·17	0·07	1·28	22·04
D	—	0·01	0·02	0·01	0·04	0·01	0·09	1·55
E	0·04	0·03	0·01	—	—	—	0·08	1·38
F	0·02	—	—	—	—	—	0·02	0·35
G	—	—	—	—	—	—	—	—
Total	0·97	1·44	0·90	1·37	0·53	0·60	5·81	100·00

(*Received May 4th*, 1926.)

NOTE ON THE PRESERVATION OF PLATE CULTURES OF FUNGI

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(With 2 Text-figures.)

Most pathologists appreciate the need for keeping records of fungal growths obtained during the course of their investigations, and many have preserved plate cultures by treatment with formaldehyde vapour

Fig. 1.

and sealing with wax, only to find such records unwieldy and expensive. To reduce expense and storage room, the following method of preserving plate cultures has been devised and has proved to be reasonably satisfactory.

The fungus should be grown on an agar or gelatine medium of such composition as will give a typical growth, but one which is not too

dense, and the plate culture should be used before the growth has developed to within half an inch of the edge. The culture may be killed by placing a filter paper moistened with formaldehyde in the lid of the Petri dish, and leaving the closed dish in an inverted position over night. Next day the lid is removed, the culture well covered with hot water, and placed in the steamer until the agar has dissolved. The fungal growth can be floated off into another dish of boiling water, and

Fig. 2.

steaming continued until the growth is freed from agar. Usually three hours' steaming is sufficient. The growth can then be floated on to a clean piece of glass (old photographic plates are satisfactory) and allowed to dry. When dry it is covered with another sheet of glass of similar size, and bound after the fashion of lantern slides.

Three difficulties may arise. (1) The growth may be too dense. (2) It may be of such a nature that the agar is removed with difficulty. (3) The growth may split badly in drying. The first may be overcome by choosing a medium which will give a thin growth; the second is

partially overcome by prolonged steaming; and the third by slow drying and previous treatment of the mounting glass with egg-albumen used in microtome work.

Fig. 1 shows a culture of *Thielavia basicola* mounted in this way. Fig. 2 is a photomicrograph taken from a similar culture and shows the masses of chlamydospores *in situ*.

(*Received July 15th*, 1926.)

A STUDY OF TWENTY-FOUR STRAINS OF *ACTINOMYCES* AND THEIR RELATION TO TYPES OF COMMON SCAB OF POTATO

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(With Plates XV–XX and 2 Text-figures.)

CONTENTS.

INTRODUCTION.

In a previous paper(14) attention was directed to the fact that various strains of *Actinomyces*, pathogenic to potatoes, showed differences in culture, which could not be accounted for if all the strains were to be regarded as belonging to the single species *Actinomyces scabies*. In the absence of any detailed cultural examination, the so-called "strains" were assumed for the time being to fall into a hypothetical "*Actinomyces scabies* group" but the suggestion was made that they might be distinct

A grant in aid of this publication has been received from the Ministry of Agriculture and Fisheries.

species each able to produce "scab." It may now be pointed out that in addition to the cultural differences existing among the strains, an even greater discrepancy occurred between the strains as a group and the original scab-producing organism (*Oospora scabies*) as described by Thaxter (21). This description is brief, and in view of subsequent knowledge of the *Actinomyces* group, is of little use for purposes of exact comparison. *Actinomyces* cultures in general are extremely sensitive to cultural methods, and undergo changes which may render them unrecognisable. Thus, Thaxter's description of his cultures on the empirical media in common vogue at the time forms no accurate definition of his species. In one particular, however, the description is valid, for it includes the characteristic that the growth of the organism on ordinary nutrient media is accompanied, when in direct contact with the air, by a dark stain, which diffuses through the medium. On potato agar, this stain becomes smoky black and almost opaque. In this respect several of Millard's cultures (14) differed from Thaxter's (21). Only one out of five developed a stain on protein media which could be described as "dark," and three produced practically no stain at all. This chromogenic character of the original scab organism was apparently so distinctive that subsequent investigators appear to have been reluctant to consider any *Actinomyces* type as causative of scab unless it exhibited the peculiarity. Thus, Lutman and Cunningham (12), in their pioneer work on potato scab, say, "This browning is of much value in identifying the organism when isolating it from potato scab, soil and other sources," and, again, "This browning is more or less marked on all strains known to produce potato scab." It would seem that these authors attached so much importance to this characteristic that they sought to minimise other noteworthy cultural differences between their strains, and ultimately referred all to Gasperini's *A. chromogenus*. It is an interesting fact, however, that even in this early work on potato scab various non-conformable "strains" of the causative organism had to be acknowledged.

Subsequent work by Krainsky (7), Waksman and Curtis (22), Conn (3) and Waksman (24) has shown that this brown pigment formation is not in itself a specific character, and indeed that *A. chromogenus* is a group of numerous species. Thaxter's (21) original description of the organism, however, still held, and this pigment formation was regarded as an essential feature of any strain of *Actinomyces scabies*. Waksman (25) retains it as a key character for the identification of his species, but notes that a number of cultures received from other investigators showed distinctive differences from his own *A. scabies* type, "particularly as to

the production of a brown soluble pigment on organic media and aerial mycelium on synthetic media."

Another character which has come to be regarded as typical of *A. scabies*, is that of producing a black stain in tyrosin media, but, here again, some accredited strains produce no stain and others vary in the intensity with which they produce it.

The causal organism of common scab is thus ill-defined, and the suggestion that the species should be widened to an *A. scabies* group consisting of various strains is but a temporary expedient. The reasons for this confusion are not far to seek. The minute size of the organisms renders the demonstration of morphological characters a matter of extreme difficulty, and every recent investigator has observed the extreme variability of the organisms in culture. Without some standardised cultural technique the task of defining any species by its characters on artificial media is practically hopeless.

Further, scab lesions are often so different in appearance that it is difficult to believe that they are symptoms of one disease. The questions arose (*a*) whether more than one species of *Actinomyces* could excite scab, and (*b*) what is the relation (if any) between the specific organism and the type of scab produced.

In attempting to answer these questions it was necessary to secure different strains of *Actinomyces* from potato scabs of varying types, to prove the pathogenicity of these strains by means of inoculation experiments, and to make accurate records of the scab produced by each. This being done, it was essential to develop a technique by means of which the characteristics of the strains might be determined.

Whilst the present investigation was in progress, work on similar lines has been published by Wollenweber (31). The conclusions of this author are in agreement with ours, but the experimental data upon which they are based leave much to be desired. Again, the *Actinomyces* species isolated by this author are too scantily defined for us to make any accurate comparison of them with our own, and we are unable to say whether any of them are identical with those herein described.

SOURCES AND ISOLATION OF THE VARIOUS STRAINS.

Twenty-four pure strains of *Actinomyces* were isolated from the sources given in Table I.

Table I.

No.	Source
1.	Scab, small, unruptured[1]. Variety of potato, Dalhousie.
2.	,, ,, ,, British Queen.
3.	,, ,, ,, Great Scot.
4.	,, large, ruptured, common type. Variety of potato, British Queen.
5.	,, medium ,, ,, ,, Up to date.
6.	,, ,, ,, ,, ,, Great Scot.
7.	,, small, unruptured, ,, ,, ,,
8.	,, medium, ruptured, ,, ,, King Edward.
9.	,, ,, ,, pitted type. ,, Kerr's Pink.
10.	,, ,, ,, common type. ,, Great Scot.
11.	Heap of decaying grass.
12.	Pot of soil and grass inoculated 4 months previously with Strain 4.
13.	Sour soil at a depth of 1 in. from a Leeds garden.
14.	,, ,, ,,
15.	Peat soil from a moorland farm where scab on potatoes is unknown. *p*H of soil = 7·1.
16.	,, ,, ,, ,, ,,
17.	Scab, ruptured, pitted. Variety of potato, Ally.
18.	Limed plot, Field 40, University Farm, Garforth. *p*H = 6·6.
19.	Scab, large, ruptured, common type. Variety of potato, Great Scot, grown in the soil from which Strain 18 originated.
20.	Scab, large, unruptured, knob-like. Variety of potato, Ally.
21.	Scab, large, slightly ruptured. Raised. Smooth. Variety of potato unknown —ex Germany.
22.	The same scab as that from which No. 21 originated.
23.	Scab, small, partially ruptured. Common type. Variety of potato, King Edward.
24.	The same scab as that from which No. 23 originated. This strain was used in the inoculation experiments but its cultural and morphological characteristics were not worked out.

It was at first intended to confine our work to Strains 1 to 10, but for various reasons these were considerably added to. It seemed desirable to test some apparently saprophytic forms of *Actinomyces*, and Strains 11 to 16 were therefore included. Again, Strain 18 was found to be present in great numbers in a certain soil which produced a badly scabbed crop, and Strain 19 was isolated from one of the tubers of this crop. It was thought that the two strains might be identical, and that a comparison of them in culture would be of interest. In 1921, two well-defined types of pitted and raised (or knob-like) scab came to our notice, and from these, Strains 17 and 20 were isolated (Fig. 3 *a* and Fig. 4 *a*). Strains 21 and 22 were derived from

[1] In the initial stages of scab formation before cork has developed, the "scab" is merely a small area of soft brown rot in which the skin of the tuber is unbroken. The term "unruptured" refers to this early stage.

scabbed potatoes in a consignment from Germany[1]. The scab was of a raised type, but obviously different from knob-like scab. The same type, which we have named "tumulus," has since been frequently seen on English-grown potatoes (Fig. 4 *b*). Strains 23 and 24 were derived from a shallow-pitted pock-like scab (Fig. 4 *e*) which was especially dominant in the wet seasons of 1923–4.

In making isolations from scabs, the tubers were first immersed in a 0·1 per cent. solution of mercuric chloride for one hour. They were then plunged for a second into absolute alcohol, since, by so doing, the water was removed from the surface and the skin of the tuber rapidly dried. This permitted of the inoculum being taken from the scab with little risk of contamination. When inoculum material is taken from young unruptured scabs, the chances of obtaining the organism are much greater than where it is drawn from an old ruptured scab. In the former case, it is a common experience to obtain plates with *Actinomyces* colonies apparently of one type, whilst, in the latter, obviously different *Actinomyces* types may occur and one can only select the dominant type, which may or may not prove to be the causative organism. In either case, bacteria are generally present. Where more than one *Actinomyces* type appears on any isolation plate, two possibilities arise: (1) that one or more types are saprophytic organisms which have introduced themselves into the scab debris, and (2) that the scab is caused by a combination of two or more parasitic types. In earlier isolations, the second possibility was ignored since, it introduced a complication to work already laborious. Exceptions were made for special reasons with Strains 21 and 22 and Strains 23 and 24, each of these pairs being drawn from a single scab. Thus Nos. 21 and 22 were cultured from a plate poured from a "tumulus" scab where, it was difficult to decide which of the two was dominant. Nos. 23 and 24 were likewise cultured from a young scab where, it did not seem likely that contamination with saprophytic types would have occurred. Both appeared on the same isolation plate, and were of interest in that, whilst No. 23 stained the medium deeply, No. 24 produced no stain. In the case of each pair, the strains were tested both singly and together in the inoculation experiments. Certain other inoculations were made in which two and even three different types were used together as the inoculum. Wollenweber(31) states that he obtained pure cultures of "scabbing" *Actinomyces* species from the grayish white aerial mycelium appearing on the surface of a scab when the potato was first lifted or after it had been kept for a short time in a warm moist chamber.

[1] Specimens kindly sent by Mr W. N. Evans of the Ministry of Agriculture and Fisheries.

This is a quick and easy method and in the case of a young unruptured scab the culture yielded may be that of the causal organism. In broken scabs, however, saprophytic species may be obtained. We have invariably taken inoculum from a more or less deeply seated part of the scab tissue.

It often happens that different *Actinomyces* types show no cultural differences on the medium used for plating out, or that such differences do not appear until after two or more weeks. In order to ascertain whether the colonies appearing were of one or more types, half a dozen were picked off and grown in slope cultures on some other media for comparison. The medium used throughout for the original isolations was nutrient potato agar made to the formula given in the media list on pp. 595–596. This gives a rapid and luxuriant growth of the various *Actinomyces* species, and although it is not the best for discerning cultural differences, it was found very serviceable.

PATHOGENICITY OF THE STRAINS.

With the exception of the illustrations in Wollenweber's (31) recent monograph, no photographs of artificially produced scab appear to have been published since those of Thaxter (21). References to different types of scab have, however, been frequently made. Bolley (1) says "...it is hard to draw the line between the surface and deep form of the disease." Lutman and Cunningham (13) say "It is barely possible that the surface and deep forms may be caused by different strains of the same organisms," and these writers give illustrations of naturally produced potato scab which differ very considerably from those of Thaxter's (21). So widely different was Thaxter's photograph of what he himself called "Deep scab" from the commonly occurring superficial scab in this country, that it was not recognised as the same disease for many years and passed into our literature as "American scab."

It is not difficult to understand this lack of illustration. Inoculation experiments with *Actinomyces* often yield disappointing results. First, there is the possibility that the organisms may have lost their pathogenicity after cultivation on artificial media. Next, where the method of soil inoculation is employed, it is difficult to know what period of time is necessary for an organism, whose vegetative habit in culture is to form compact masses of growth, to impregnate any given quantity of soil. Lastly, the conditions necessary for parasitic action of any one strain of *Actinomyces* may be unfavourable and even inimical to other strains. The question of soil temperature and moisture content

must be taken into account. In our experiments, repeated trials have had to be carried out with a number of the strains before the right conditions for infection were obtained. Throughout this work, only those strains which, under rigid experimental conditions, have reproduced definite scabs are recognised as scab exciting organisms.

The pathogenicity tests were carried out by the following two methods.

Method 1. *Direct inoculation of the growing tuber.* This was Thaxter's (21) original method. The soil was carefully scraped away from the plant to be inoculated until one or two stolons were uncovered bearing clean young tubers. The soil below the tuber was pressed firmly down in order to prevent the potato from sinking into it. Any soil particles adhering to the potato were blown off—a proceeding only possible in light dry soil—and it was then washed with a spray of sterile water. Clean flat pieces of broken pot were laid around the tuber, and a 4-in. flower pot previously washed and steamed and with its drainage hole firmly plugged with cotton wool was inverted over it. As the pieces of pot lay at a higher level than the stolon, the latter escaped any injury from the pressure of the pot. The surface film of water was allowed to dry off, and the inoculum, consisting of an emulsion of a culture on nutrient potato agar, was then transferred to the tuber. The pot was firmly replaced, covered with stiff black paper, and the whole heaped over with soil.

Objection is easily raised to this simple method of inoculation on the grounds that the precautions taken do not secure sterility. However desirable such a condition may be, it seems impossible to secure it and at the same time to retain the tuber in its natural state. If the surface of the tuber is sterilised with alcohol, formaldehyde, etc., there is risk of so changing its nature that it would no longer be susceptible to infection, and even if this were not the case, it is difficult to see what subsequent measures could be taken to ensure aseptic conditions during the incubation period. We thought it best to rely on control experiments for indications of accidental infection, and this decision was justified by the results.

The inoculations were of two kinds, which we have called "spot" and "scratch" respectively. In "spot" inoculation the tubers were blobbed with drops of the emulsion from a platinum loop, whilst, in the "scratch" inoculation, the tuber was first scratched with a sterile needle and the inoculum rubbed only along these scratches. Three tubers, each attached to a separate stolon either on the same or different plants, were inoculated with each strain of *Actinomyces*, two by the "spot" and one by the "scratch" method. Little value was attached to the "scratch" inoculation as a crucial test of pathogenicity, but it was thought that it might serve as a means of detecting weakly pathogenic strains. Eventually, however, in assessing the pathogenicity of any strain it was entirely disregarded.

Method 2. *Soil inoculation.* Here, the potatoes were grown in pots of sterile soil which were inoculated respectively with the strains of *Actinomyces* under examination. Unglazed pots of 12 in. diameter were used. These were filled with soil (taken from a plot on which the lime requirement was nil) and autoclaved at 130° C. for 1 hour. The seed tubers, as free from scab as possible, were sterilised by soaking in a 0·1 per cent. solution of mercuric chloride for $1\frac{1}{2}$ hours and allowed to sprout under sterile

conditions before planting. The plants were grown in a heated greenhouse which, prior to the experiments, was thoroughly sterilised, and during their course was constantly washed with an antiseptic solution. A nicotine preparation was burnt at intervals to keep out aphides and other insects. The plants were watered with sterilised tap water. With these precautions we have found that on no occasion did infection spread to the control pots, and we feel justified in assuming that the scab produced in any individual pot was due solely to the infection material added to it. Further proof, if necessary, was afforded by the different types of scab produced in the different pots.

The infection material which was found most easy of manipulation consisted of potato plug cultures, and these were used throughout the experiments except in one series of inoculations, where emulsions of nutrient potato agar slope cultures were substituted. The latter were found to be much less easy to handle. The inoculum for each pot consisted of 12 potato plug cultures which were added in two batches with an interval of one or two weeks. After being transferred to the pot and lightly covered with the moistened soil they were cut into small pieces and thoroughly mixed in to a depth of six inches.

A disadvantage in this method of inoculation is that it is difficult to obtain the degree of aeration in pots favourable to maximum development of the *Actinomyces*. In order to overcome this difficulty as far as possible, a quantity of gravel was mixed with the soil to make it more porous. Even so, the potatoes often tend to form in the lower and moister layers, and the conditions for scab formation are unfavourable to those strains which function only under very dry conditions. Where some of the strains produced no scab, there was always the possibility that this was due to the difficulty of maintaining a balance between the requirements of the plant for water and the necessity on the part of the organism for dry conditions. Against these drawbacks, however, the method has the great advantage over direct inoculation of the tuber that, by it, the temperature may be regulated. Whether this factor is of such moment as soil moisture and aeration or not, it doubtless plays an important part in scab production. L. R. Jones and McKinney (6) have shown that the optimum temperature for scab production is as high as 25° C., and Shapovalov (19) gives the optimum temperature for germination of the spores of *A. scabies* at 35° to 40° C. As a demonstration of the favourable influence of high temperature, we may of course cite the hot dry summer of 1921 and the phenomenal amount of scab in that year. In this connection, we were very fortunate in that all but two of our field inoculations were carried out in this year. The two exceptions were those of Strains 17 and 20, which were tested by direct inoculation in the wet, cold summer of 1922, and then gave negative results, whilst, when the same strains were tested in the greenhouse at a temperature ranging around 21° C., No. 17 proved to be a most virulent pathogen.

Duplicate and in some cases triplicate pot inoculations were made with each strain tested by the above method.

The inoculation experiments were carried out during the years 1921–5, the strains being tested in series as shown in Table II.

In the first series, the inoculations were carried out on potatoes grown on land which was ploughed out in 1918, and on which, so far as could be ascertained, potatoes had not been previously cultivated. The results were ignored, however, since some

of the uninoculated tubers showed natural scab. Apart from this failure, the experiment was of interest in that scab here occurred on land which, from the point of view of potato culture, was virgin soil. Series 2 was carried out in another part of the field where the soil was distinctly acid, and natural scab was rare. In addition to the single inoculations with each *Actinomyces* strain, certain multiple inoculations were carried out in which two or more strains were combined. In a few cases also, cultures of common soil bacteria were introduced with the idea that bacterial infection of the lenticels of the potato tuber might facilitate the entry of the *Actinomyces*. No confirmation of this hypothesis was obtained. Where, in Table II, the number of any strain appears in more than one series, the previous test was unsatisfactory. In these repeat tests the conditions of moisture and temperature were made to vary, and by so doing it became evident that certain strains were extraordinarily sensitive to the balance of these two factors. Thus, for example, Strain 21 was entirely negative under normal conditions of summer heat and soil moisture, whereas when the soil was allowed to dry out so much that the plants died of drought the inoculation was successful.

Table II.

Series	Time	Type of inoculation	Strains tested Nos.	Multiple inoculations Nos.
1	1921 (Aug.)	Direct—Field	1–16 (omitting 6, 12)*	—
2	„ (Sept.)	„ „	„ „	—
3	1922 (July)	„ „	17, 20	—
4	1922–3	Soil—Greenhouse†	17–20	—
5	1924	„ „	18–22	21 + 22 20 + 21 + 22
6	„	„ „	18, 20, 21	—
7	1924–5	„ „	18–24	18 + 19; 20 + 21 20 + 22; 21 + 22 20 + 21 + 22 23 + 24 18 + soil bacteria 19 + „ 20 + „

* Strains Nos. 6 and 12 had been proved in culture to be duplicates of Strains Nos. 5 and 4 respectively.

† The temperature of the greenhouse fluctuated between 10° C. and 26° C. but generally ranged around 20° C.

A list of the inoculation results is given in Table III in which notes of the virulence of the causal organisms and the types of scab produced by each are added. The degree of virulence is indicated by numbers, the maximum being denoted by the figure 12. In any comparison of virulence of the different strains the figures given register only the parasitism of any particular strain under the conditions of the experiment in which it was tested. The scab on a tuber is largely caused by the reaction of the host to the stimulus of the parasite. In the more

extreme types of scab the internal morphology shows differences which are in themselves strong evidence of attack by organisms of widely varying physiological characters and it is hoped to discuss this point in a later paper. If, however, we confine ourselves to the more intermediate forms of scab, the actual destruction of the tissues by the parasite is comparatively small, and the scab consists almost wholly of cork. Thus the *degree* of scab produced must largely depend on the age of the tuber at the time of attack, and on its subsequent growth. The variety of the potato will also probably exert some influence on the size of the scab, since some apparently produce cork more readily than others. Thus Priestley and Woffenden(17) have shown that "King Edwards" and "Majestics" lag behind other varieties in the production of wound cork, and we may correlate this statement with the fact that "King Edwards" rarely scab as badly as other varieties. We do not think that this factor affects our results, since all the varieties used scab badly. Neither is there any evidence to indicate that the *type* of scab as opposed to the *degree* of scab is in any way dependent on the variety of the potato. On the contrary, the experimental results obtained prove that different types of scab may be produced on the same variety with different strains of *Actinomyces*, and *vice versa*, that the same type may be produced on different varieties by one strain of *Actinomyces*. Thus, the same deeply pitted scab was produced by Strain 17 on both "Ally" and "Great Scot" (Fig. 3 *b* and *c*). The variety of potato may modify the intensity of the scab but does not change its type.

Various terms which are defined later are used in Table III for the description of the scab types produced. The abbreviation "A.m." stands for aerial mycelium, which is often found as a delicate grayish white weft of mycelium on the surface of the scabs when the potatoes are first lifted.

Strain No. 17 proved of marked virulence. Fig. 3 *b*, the *artificially produced* scab, should be compared with Fig. 3 *a*, the *natural* scab from which the strain was isolated. In the latter the potatoes show stages of scab which resemble the ordinary type but actually, the base of these scabs is comparatively soft, whilst, in the "ordinary" type, it consists of a thick corky layer.

It was noticed on lifting the plants inoculated with this strain that the fibrous roots were stunted and somewhat thickened, and thickly studded with irregular dark brown nodular outgrowths, on some of which the typical grayish mycelium of *Actinomyces* was present (Fig. 3 *d*). Some of the stolons were similarly affected. As far as we are aware this

is the first record of any root infection by scabbing *Actinomyces*. In this country, scab rarely appears to exert any adverse effect on yield, but, in America, a serious reduction in yield has been often reported and this may possibly be accounted for by such root infection. A comparison of Thaxter's figure of "Deep Scab" with Fig. 3 *b* leaves little

Table III.

Results of inoculation tests.

Strain of *Actinomyces*	Positive or negative	Type of scab produced	Virulence of scab
1	–	— —	—
2	+	"Pitted." Very dark. A.m. abundant	4
3	+	"Ordinary." A.m. abundant	3
4	–	— —	—
5	–	— —	—
7	–	— —	—
8	–	— —	—
9	+	"Pitted." Very dark. A.m. abundant. (Fig. 4 *d*)	5
10	+	Superficial	2
11	–	— —	—
13	–	— —	—
14	–	— —	—
15	+	"Pimple-like." Soft texture. Dark. (Fig. 3 *e*)	3
16	+	Ordinary	2
17	+	"Pitted," forming deep furrows. Very dark. Much browning of adjacent tissues. A.m. abundant. (Fig. 3 *b* and *c*)	12
18	+	Indeterminate. Slight browning of tissues	1
19	+	,, ,, ,,	1
20	–	— —	—
21	+	"Tumulus." No browning. (Fig. 4 *b*)	6
22	+	Probably positive. Indeterminate. No browning	1
23	+	"Ordinary" to "pitted." Some browning. The lids of the unruptured scabs black. (Fig. 4 *e*)	6
24	+	Raised, type not clear. No browning of tissues	1

doubt as to the identity of the two types. Correlated with this is a fairly close correspondence between the cultural characters of Strain 17 and the description of Thaxter's original scab organism. It seems probable, therefore, that the two organisms are identical and Strain 17 is thus regarded as the true representative of the species *A. scabies* (Thaxter) Güssow.

Types of scab.

As a result of the present investigation the writers find it necessary to use the following descriptive terms for the various types of common scab.

(1) *Superficial scab.* This is little more than a brownish abrasion of the skin of the potato usually small in extent but occasionally covering larger areas of the surface. The type is not very definite and may be merely a mild form of ordinary scab.

(2) *Ordinary scab.* This has been described by Millard (16) as "characterised by an irregularly concentric series of wrinkled layers of cork arranged round a central core or depression." The surface of the individual scab is rugose, and its outline irregular. This type is that most often seen on medium loams.

(3) *Pitted scab* (Fig. 3 *a*, *b* and *c* and Fig. 4 *d* and *e*). This is characterised by a pock or depression in the tissue of the potato which is seen when the covering skin of the scab ruptures. The outline of the depression is at first circular, and is bordered by the ragged edges of the torn skin. It is dark in colour, and the surrounding tissue is also generally stained. The interior of the scab during its development is somewhat soft and pulpy, and the formation of cork which ultimately lines the pit is slow. In the more virulent forms of this type the pits may become 3 to 4 mm. deep, and where two or more scabs coalesce deep furrows may be formed. Pitted scabs occurring under natural conditions are frequently inhabited by springtails which apparently feed upon the pulpy scab debris.

(4) *Stud scab.* In place of a depression in the potato tissue there is a distinct elevation or swelling 2 to 3 mm. above the surface, forming a warty outgrowth which is easily cut off with a knife. The top of the scab is smooth and more or less circular, whilst the sides rise abruptly from the surface giving a stud-like appearance (Fig. 4 *a*). The scabs are only slightly brown when the potato is lifted and the surrounding tissues are not discoloured. They are extremely corky. A close examination shows that the original skin of the tuber has been ruptured, and that in the younger scabs there is a minute depression in the centre of each marking the point of infection. A similar point has been noted in connection with the ordinary type. Thus, in early development, at least, the mode of formation is analogous to that of other scabs. This type appears to be very similar to Wollenweber's (31) "Buckel" scab.

(5) *Tumulus scab.* This type is similar in nature to stud scab, and

externally, the difference between mature scabs of the two kinds is more of degree than of type. In the earlier stages of development a cone-like depression is first formed (Fig. 4 *c*) which is easily distinguished from pitted scab by the firm corky layer lining it. Later, the scabs are pushed upwards and assume a mound-like appearance, which differs from that of stud scab in that the sides of the mound are sloping and do not rise abruptly from the tuber surface (Fig. 4 *b*). Tumulus scab is not so raised as stud scab, and this difference is due to its manner of origin. Thus, in the former, a decided sinking or pitting of the tissues first takes place, whilst in the latter, the initial attack consists of a pin point hole and swelling of the tissues follows almost at once. A somewhat misleading feature of the photograph in Fig. 4 *c* is that the scabs there appear much darker than they really are.

(6) *Pimple scab* (Fig. 3 *e*). This is not a well-defined type, and has only been met with in inoculation experiments. The scabs are small, soft, and pimple-like, but it is possible that with further growth they would prove to belong to one of the other well-defined types.

Recovery of inoculated strains from the artificially produced scabs.

In every positive case re-isolations were made from the artificially produced scabs, and excepting Nos. 2 and 3 all the strains were recovered, and found to agree with their respective originals.

In the case of Strains 2 and 3 the tubers had been frosted, and rotted so quickly that no isolations could be made. The two strains are, however, included as positives in Table III.

Twelve strains out of the 24 tested thus proved to be scab producers, and, as will be shown later, 11 of these are distinct species. There can be little doubt that this number of scab-producing species will be augmented by subsequent investigators.

Discussion of results.

(*a*) *Occurrence of different types of scab in nature.* Observation of scabby potatoes indicates that the scab on a single tuber, and even on a whole crop, is frequently all of one type. This is especially noticeable in a season marked by extremes of heat, dryness, or humidity, and this fact supplies the explanation of the phenomenon. Inoculation experiments have shown how dependent is the parasitism of *Actinomyces* species upon a certain balance of temperature and humidity. There would seem to be a comparatively small range within which these two factors are in just that agreement which accords with parasitic attack

on the part of any given species, and natural infection is no doubt controlled in this way. Soil reaction and soil texture will exert a further selective action, and thus, under certain extreme conditions either of climate or soil, tubers are parasitised by one species or one cognate group of species to the exclusion of others.

(*b*) *The occurrence of scab on virgin soil.* The case of a scabbed crop occurring on ploughed out grass where potatoes had not been grown for over 50 years has been mentioned. Instances of this kind appear to be not uncommon (2, 5, 11). Such cases can scarcely be accounted for on the basis of one obligate scab-producing species, even when we admit the suggestion of Shapovalov (20) and Lutman (11) that *A. scabies* may exist saprophytically for a time in the soil. Where, however, as has been shown, a number of species of *Actinomyces* (two of which, Nos. 15 and 16, were natural saprophytes) can attack the potato tuber, it is easy to see how scab may arise on land never previously planted with potatoes.

(*c*) *The incidence of scab.* In any consideration of the incidence of scab, the physiological characteristics of many species must now be included. Certain investigators assert that the primary factor in the occurrence of scab is soil reaction, and have tried to correlate the latter with the range of hydrogen-ion concentration within which a few "strains" of *A. scabies* develop. Such a correlation can obviously hold only for the particular species of scabbing *Actinomyces* present in the soil under consideration, and not for those which thrive in other types of soil.

CULTURAL CHARACTERISTICS.

Every investigator of the Actinomycetes meets the difficulty that the characters of any species are liable to undergo wide variations in culture. These may consist in the loss or acquisition of certain attributes such as power of spore production on some or on all media, characteristic smell, pigment production, etc. Lieske (8) has shown that anaerobic strains may become aerobic, and that the temperature range may be altered. He also shows that from a culture normally bearing a sporogenous layer, two strains may be drawn, one sporing and the other non-sporing. Waksman (26) says "ordinary bacteriological methods will not hold when applied to this group of micro-organisms," and again "When the same culture grown on different artificial culture media for a period of time, and often even for one short generation is studied morphologically and physiologically, notable variations are observed, so that the untrained observer would be apt to take these as representing different forms." Conn (3) says "It has proved impossible to identify

with certainty any organism thus far studied with any species described by Krainsky (7), and again that "every new medium that has been tested has served to break up still further the types already recognised by the appearance of their growth on other media."

We are convinced that this apparent cultural inconstancy is due to lack of technique rather than to any inherent eccentricity on the part of the organisms. We have succeeded in keeping the cultures of all the strains investigated constant on a great variety of media for a long period of time, extending in some cases to 4 years. Much of our technique is well known, but the details are so vital to any proper study of the group that some account of it may be excused.

One of the most potent factors in the culture of *Actinomyces* is the chemical composition of the media. Where good brands of foodstuffs were selected and adhered to, and the materials accurately weighed out, some of the ordinary nutrient media were found to give constant and useful diagnostic results. Of greater service were the synthetic media introduced by Krainsky (7) and Waksman (23–30).

The reaction of the media is of equal importance. Standardisation was made by the Clark and Lubs colorimetric method, and in comparing one strain with another or in repeating any strain, the same initial *p*H of the medium was employed.

The method of sub-culture is of great moment, and we have, like Lieske (9), found that the manner of growth, especially in the initial stages, varies considerably with the inoculum taken. This is particularly noticeable in liquid media where an inoculum of the vegetative stroma tends to sink to the bottom of the tube and produce "bottom" growth only, whilst one of the aerial mycelium will float on the surface and give "surface" growth. The inoculum taken should be uniform in nature and in quantity throughout the sub-cultures made. The original inoculum should not be left adhering to the slope, but shaken into the water at the bottom of the tube or removed. Where a sub-culture is taken from a medium on which a pigment has been produced, it may happen that the pigment from the inoculum will diffuse into the new medium and thus give a fictitious pigment. Special care must be taken in inoculating liquid media, and it is advisable first to rub the inoculum on the sides of the tube at the surface of the liquid before shaking it into the medium. Surface growth is often obtained in this way where otherwise only bottom growth would appear.

The medium for stock cultures should give an abundant growth, and, if possible, produce aerial mycelium. Nutrient potato agar was even-

tually selected for this purpose. On this medium all the strains produced both vegetative and spore growth excepting Nos. 13, 14, 15, 17 and 23. Stock sub-cultures were made at regular intervals of one month. After varying lengths of time some of these cultures showed a lack of vitality which evinced itself in a falling off of spore production, or in loss of pigment formation. When this occurred, the culture was replated and new cultures made. Not all the colonies obtained by this practice show a complete return of vigour. Thus, it was found that a culture producing a deep stain on protein media may, when plated out, give certain colonies which stain as deeply as the original culture and others which do not stain at all. A number of new cultures must be prepared and a selection made. All our stock cultures were replated in this way from time to time.

The media selected for delineation of characters are given in the following list. Many of these have been taken as they stand from Waksman's (27) list, but, as some slight changes have been introduced in others, formulae have been given throughout. The hydrogen-ion exponent of each medium is also stated. Pure chemicals were used in all cases.

CULTURE MEDIA.

Solid media.

1. Saccharose synthetic agar (based on Czapek's solution modified).
   K_2HPO_4 1 gm.; $MgSO_4$ 0·5 gm.; KCl 0·5 gm.; $FeSO_4$ 0·01 gm.; $NaNO_3$ 2 gm.; saccharose 30 gm.; distilled water 1000 c.c.; agar 15 gm. pH = 6·6.
2. Glycerine synthetic agar.
   Similar to No. 1 excepting that glycerine replaces saccharose. pH = 6·7.
3. Dextrose synthetic agar.
   Similar to No. 1 excepting that dextrose replaces saccharose. pH = 6·8.
4. Calcium malate glycerine agar (Conn's (3)).
   Calcium malate 10 gm.; NH_4Cl 0·5 gm.; K_2HPO_4 0·5 gm.; glycerine 10 gm.; distilled water 1000 c.c.; agar 15 gm.
   Reaction adjusted with NaOH to pH = 7·0.
5. Dextrose agar (Krainsky's (7)).
   Dextrose 10 gm.; K_2HPO_4 0·5 gm.; asparagin 0·5 gm.; distilled water 1000 c.c.; agar 15 gm. pH = 6·8.
6. Tyrosinate agar.
   Dextrose 10 gm.; tyrosin 1 gm.; $(NH_4)_2SO_4$ 0·5 gm.; K_2HPO_4 0·5 gm.; distilled water 1000 c.c.; agar 15 gm.
   Reaction made approximately neutral with NaOH. pH = 6·9.
7. Nutrient potato agar.
   500 gm. peeled potato; 10 gm. Fairchild's peptone; 10 gm. Liebig's extract; 5 gm. NaCl; 15 gm. agar; 1000 c.c. tap water.
   The potato is cut into small cubes to which 350 c.c. of water are added and

the whole steamed for three-quarters of an hour. It is then strained through fine muslin without squeezing the pulp. The other nutrients are dissolved in 350 c.c. of water which is then added to the potato decoction, and the whole steamed for three-quarters of an hour. The mixture is then made up to bulk, standardised and filtered, after which the agar is added. pH = 7·0.

8. Egg albumen agar.
   Dextrose 10 gm.; K_2HPO_4 0·5 gm.; $MgSO_4$ 0·2 gm.; $Fe_2(SO_4)_3$ trace; egg albumen 0·15 gm.; distilled water 1000 c.c.; agar 15 gm.
   1·5 gm. of egg albumen was first dissolved in about 100 c.c. of water and made neutral to phenol-phthalein with $N/10$ NaOH. One-tenth of this volume was then added to the warm solution of the other ingredients.
9. Gelatine.
   Gold label gelatine 150 gm.; distilled water 1000 c.c.
   Reaction adjusted with NaOH to pH = 7·4.
10. Potato plug.
11. Carrot plug.
12. Starch nitrate (for diastatic action and reduction of nitrate).
    Formula as for medium 18 with the addition of 1½ per cent. agar.

Liquid media.

13. Saccharose synthetic solution.
    As for No. 1. pH = 6·7.
14. Glycerine synthetic solution.
    As for No. 2. pH = 6·7.
15. Dextrose synthetic solution.
    As for No. 2. pH = 6·2.
16. Glucose broth.
    Glucose 10 gm.; peptone 10 gm.; Liebig's extract 5 gm.; NaCl 5 gm.; distilled water 1000 c.c.
    Reaction adjusted with NaOH to pH = 7·1.
17. Nutrient broth, prepared in the usual way.
    Reaction adjusted with NaOH to pH = 7·2.
18. Synthetic solution (for starch hydrolysis).
    Starch (Lintner's soluble) 20 gm; K_2HPO_4 1 gm.; $MgSO_4$ 0·5 gm.; KCl 0·5 gm.; $NaNO_3$ 2 gm.; $CaCO_3$ 2 gm.; distilled water 1000 c.c.
    The starch was made into a paste and boiling water poured on it. This when steamed for an hour gave a clear solution. The salts were then added and the solution made up to the required volume. Small Erlenmeyer flasks were used for the cultures, 85 c.c. of the solution being poured into each.
19. Brom-cresol milk.
    This was prepared according to the formula given by Clark and Lubs (33). The medium was sterilised by steaming for 20 minutes on three successive days.

Observation reveals detail in cultures of the *Actinomyces* which is constant and characteristic for any given species. The manner of growth on solid and in liquid media, the mode of formation of aerial mycelium,

colour changes in the vegetative and reproductive parts and pigment production in the medium, constitute a wealth of diagnostic data.

The point of greatest importance is that the cultures undergo a sequence of changes during their period of growth. This period varies somewhat with the medium, but, more generally, with the particular species. Thus, some species (*e.g.* No. 20) reach maturity in a week or 10 days, after which the cultures remain practically constant, but other species (*e.g.* No. 9) take much longer, and others (*e.g.* No. 18) reach maturity only after 4 weeks. The character of growth passes through gradations in a regular and constant sequence, and the final appearance of a culture may be totally different from that of its earlier stages. Where these progressive changes are noted, the recognition of any species is a comparatively simple matter, and so definite are the characters that it becomes unnecessary to use more than a moderate number of media for their delimitation.

The cultures on all media were duplicated whereby the constancy of each character or gradation could be noted. With the exception of gelatine all were incubated at a temperature of 23°–24° C. Gelatine cultures of Strains 1 to 16 and also 21–23 were kept at a temperature 15–20° C., and the remainder at 23° C. All cultures were kept for 4 weeks and records made at weekly intervals. Excepting where, in a few cases, no parallel could be found, colours are as described by Ridgway (18) and are distinguished throughout the descriptions by inverted commas.

MORPHOLOGY OF THE STRAINS.

The morphological characters of the *Actinomyces* group have been fully worked out by Drechsler (4). In the present work we have confined ourselves to ascertaining what, if any, were the morphological differences between our different strains which could be demonstrated with a magnification of 1087. Drechsler's technique was followed throughout with the exception that "impressions" were taken from Petri-dish instead of slope cultures since, in this way, less mutilation of the conidiophores took place. The results of the examination will be found in Figs. 1 and 2 and in the description of the strains. The terms "split" and "attenuated isthmus" as applied to the septa are taken from Drechsler (4).

It is only necessary to point out certain peculiarities in some of the strains. Strains 13 and 14 produce no aerial mycelium or only a trace on any media. Each, however, exhibits the peculiarity of forming colonies on various media in which a raised central dark spot occurs.

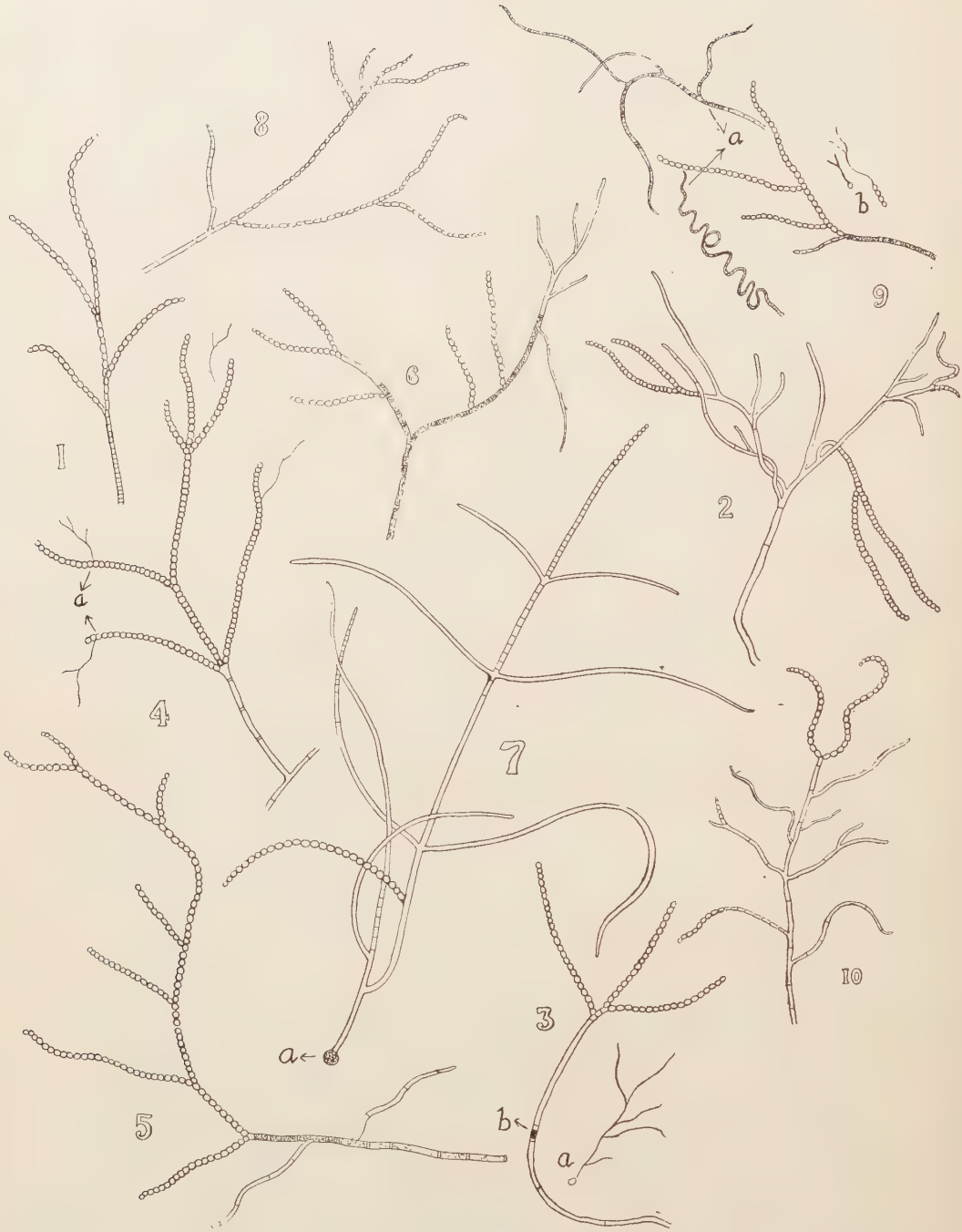

Fig. 1. Conidiophores of Strains 1–10 as numbered. Characteristic "split" septa are seen in Nos. 4, 5, 6 and 9, and germinating conidia in Nos. 3 *a*, 4 *a* and 9 *b*; 3 *b*, metachromatic granule. 7 *a*, germinating chlamydospore. 9 *a*, whip-like segments.

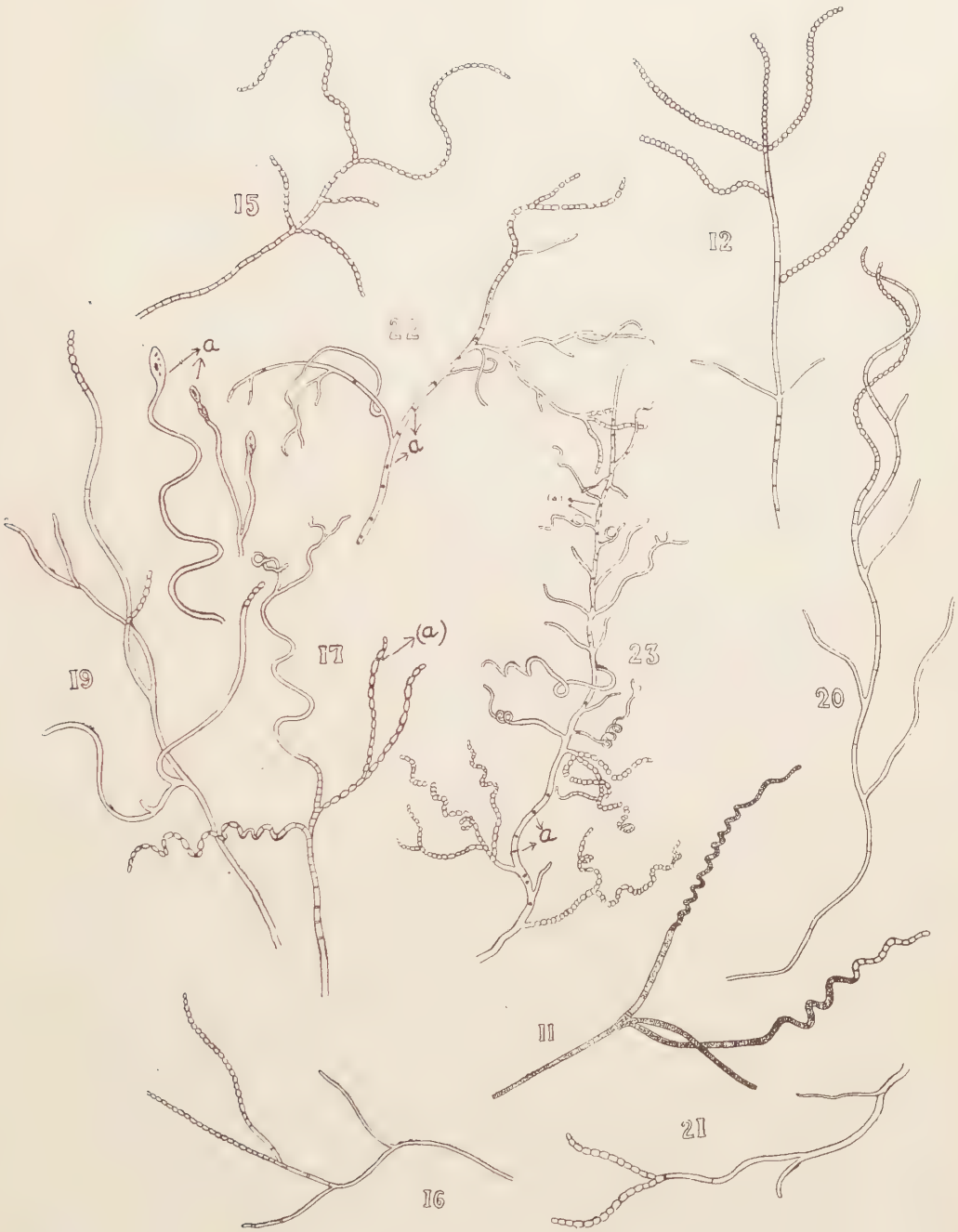

Fig. 2. Conidiophores of Strains 11–23 (omitting Nos. 13, 14 and 18). "Split" septa are seen in Nos. 11, 12 and 16, and "attenuated isthmus" septa in No. 17 *a*; 22 *a* and 23 *a*, meta-chromatic granules; 19 *a*, club-like structures containing meta-chromatic granules.

These points of growth were found to consist mainly of small masses in which short chains were present. The spores, although smaller in dimensions than those of the other strains, were of greater diameter than the hyphae of the vegetative mycelium, which, in both strains is extremely minute. Nothing in the nature of a conidiophore could be detected. Although, therefore, some simplified fructifications may have been present which could not be demonstrated, we are inclined to consider such spore masses as colonies of chlamydospores.

Similar dark-centred colonies occurred on old cultures of Strains Nos. 9, 10 and 15, noticeably on tyrosinate agar, but an examination of those in No. 9 showed them to consist of swollen vacuolated hyphal segments.

In Strain 19 one or two club-shaped structures were found (Fig. 2) reminiscent of *A. bovis* but, as the cultures were several weeks old, they may have been involution forms.

CONCLUSIONS.

Two pairs of duplicates Nos. 5 and 6 and Nos. 4 and 12 appeared among the 24 strains, and in each of these pairs, the cultures of the two strains on all media were identical. This correspondence was significant since it provided a confirmation of the stability of our technique. A third pair of strains arose in Nos. 18 and 19, but here anomalies occurred, which, although insufficient to separate the strains, indicate that cultural characters in the *Actinomyces* may be modified by their previous mode of existence. They are here regarded as variants of one species.

The remaining strains must be considered as distinct species.

Comparison of these species has been made with those described in Waksman's (23–30) monograph, but no agreement has been found. In a newly examined group of organisms already known to be rich in form the number of species is probably legion. The method of describing "species groups" rather than individual species will hinder rather than help the demarcation of species in the genus, and, in our opinion, any attempt at grouping the species should follow and not precede their exact definition. Waksman (29) states that "if attempts were made to describe all the details of the cultures and to make new species based on some variations from others, the 40 or so species which he has described could have been easily increased to several thousand." He accepts "variability" as an unavoidable attribute of the cultures for which "due allowances" must be made, and gives an example of the variation allowed in a "species group." This in itself is sufficient to

show us that we could not hope to find parallels with our own species. With the exception then of Strain 17 referred to the species *A. scabies* (Thaxter) Güssow, the species herein described must be considered as new and have been named as follows:

Strain		Strain	
1	*Actinomyces carnosus*	14	*Actinomyces salmonicolor*
2	,, *Setonii*	15	,, *Wedmorensis*
3	,, *marginatus*	16	,, *coroniformis*
4, 12	,, *praefecundus*	17	,, *scabies* (Thaxter) Güssow (emend. Millard & Burr)
5, 6	,, *tenuis*		
7	,, *gracilis*	18, 19	,, *clavifer*
8	,, *Sampsonii*	20	,, *praecox*
9	,, *viridis*	21	,, *flavus* (Millard & Burr)
10	,, *Loidensis*	22	,, *craterifer*
11	,, *spiralis*	23	,, *fimbriatus*
13	,, *maculatus*		

The description of these species preceded by explanatory notes is given and is followed by a key for their identification.

EXPLANATORY NOTES TO THE DESCRIPTIONS.

Solid media. In the case of all solid media excepting gelatine, potato and carrot plug, the descriptions refer to agar slope cultures.

Colour of streak. This refers to the vegetative stroma and where the colour is noted, it has generally been observed from the upper surface; in some cases, however, where the aerial mycelium appears almost simultaneously with the vegetative growth, the colour of the latter has been taken from the reverse side of the culture.

Pigment. This refers to the colour of the stain which diffuses through the medium from the growth. In liquid cultures the pigment when produced is almost invariably associated with surface growth, and diffuses downwards as in solid media. One or two cases occurred, however, where, in the absence of surface growth, the pigment arose from bottom growth and diffused upwards through the liquid. It is unfortunate that there appears to be no colour in Ridgway (18) corresponding to the frequently occurring golden brown of the pigment, and we have thus been obliged to retain the common terms in such cases. Absence of pigment is not noted.

Guttation. This phenomenon, in which small droplets of a clear and highly refractive fluid are exuded from the surface of the culture, is exceptionally common in the *Actinomyces*. When present it may be copious or sparse, and the drops may be distributed over the whole surface or confined to certain parts of it, such as the margins. The droplets may be coloured or colourless. A marked peculiarity of the phenomenon as associated with the *Actinomyces* is that where, the drops of liquid dry up, as they often do, the pin point holes in the growth from which they have exuded become the centres of shallow crater-like depressions which are very striking in appearance. This is especially the case in *A. craterifer*. The absence of guttation is not noted.

Brom-cresol milk cultures. The original colour of the brom-cresol milk after sterilisation was "Light Medici Blue." Cultures were incubated at 24° C. for 38 days,

after which they had reached a point at which peptonisation was complete in the greater number. The colours noted are in every case those seen by reflected light. Clotting, as understood in bacterial cultures, scarcely occurred at all, but a softly granular precipitation of the casein took place, and the term coagulation must be taken to mean this. Coagulation was quickly followed by digestion of the clot giving an upper zone of more or less clear liquid. This zone was measured as from the original surface of the medium. The colour changes in the medium generally denoted a change in reaction towards alkalinity, but, in some cases, the reaction was masked by some other pigment. It was, therefore, considered best to register all colour changes by the Ridgway (18) standards rather than to suggest the degrees of alkalinity arrived at. Two and even three distinct zones of colour were often produced in the medium apparently corresponding to the degree to which coagulation and digestion had gone but only the colour of the upper layer, the digestion zone, has been given.

Tyrosinase reaction. A "positive" reaction signifies the production of melanin stain. Considerable variation in the intensity of this stain was found to occur with tyrosin from different sources. Weak pigments other than melanin were sometimes produced in the medium, and have been noted.

Anaerobic growth. Anaerobic tests were made in Buchner tubes using nutrient potato agar cultures. Growth appeared only with Strain 13, where it became evident in each of the duplicate tubes after one week.

Starch hydrolysis and nitrate reduction. These tests were first carried out with the liquid media, No. 18, and for starch hydrolysis this proved very satisfactory. The relative speed and degree of hydrolysis varied greatly and the cultural characters exhibited were strongly opposed in the different species. The nitrite was estimated after 4 weeks by the Griess-Ilosvay method, but it was found that the control flasks gave positive reactions. Repeat tests confirmed this observation. Although, therefore, many investigators appear to have used this test, it was clearly impracticable under our laboratory conditions. Tests were then made with nitrate plates, but, here again, the control plates only remained free from nitrite for 1 week and the tests were necessarily carried out at that age. It must be borne in mind that the estimation of nitrite is one of nitrite accumulation rather than of nitrite production, since the nitrite formed may be re-assimilated by the cultures. The amount of nitrite present was shown by the breadth and intensity of the coloured zone after treatment with acidified potassium iodide, and this is indicated by numbers with a maximum of 10.

Gradational changes in colour, production of aerial mycelium, etc. The numbers used to indicate these changes often approximate to but do not accurately register *weekly* changes. A.m. = aerial mycelium.

Growth at 37·5° *C.* The cultures at this temperature showed little characterisation and are, therefore, not described; the peculiarity that none of the cultures produced aerial mycelium should, however, be noted.

DESCRIPTION OF THE SPECIES.

Strain 1 = *Actinomyces carnosus.*

Morphology.

Conidiophore. Simple, branched, the whole apical portion closely septate.

Conidia. Cylindrical. $1{\cdot}0 \times 0{\cdot}75\mu$. (Fig. 1.)

Cultural characteristics.

Solid Media.

Saccharose synthetic agar. (Fig. 5 A.)

Colonies—umbonate, fleshy. *Streak*—(1) fair; (2) good. "Pale Smoke Gray."

A.m.—abundant. (1) "Drab Gray"; (2) "Smoke Gray." Appears early.

Guttation—copious minute colourless drops over whole surface.

Pigment—"Cream" after 4 weeks.

Glycerine synthetic agar. (Fig. 5 B.)

Streak—develops slowly, good, wrinkled. "Pale Smoke Gray."

A.m.—scant, white.

Pigment—light golden brown turning to "Buff Pink."

Dextrose synthetic agar. (Fig. 6 A.)

Streak—good, acervate, echinate. "Pale Olive Gray." Margins crenate.

A.m.—abundant. (1) White; (2) "Smoke Gray" with white patches.

Guttation—copious minute colourless drops over whole surface.

Pigment—(1) "Ivory Yellow"; (2) "Cartridge Buff."

Calcium malate glycerine agar.

Colonies—umbonate; edges flat, crenate. *Streak*—(1) poor; (2) fair. "Pale Smoke Gray."

A.m.—scant, confined to centres of original colonies. (1) White; (2) "Smoke Gray."

Pigment—"Ivory Yellow"—late.

Dextrose agar (Krainsky).

Colonies—umbonate. *Streak*—fair, acervate. "Pale Smoke Gray."

A.m.—(1) scant; (2) abundant. (1) White; (2) "Smoke Gray."

Guttation—copious, mainly along edges of streak.

Nutrient potato agar. (Fig. 6 C.)

Colonies—very large, umbonate, fleshy, lustrous, very raised centres and lobe-like puckered margins. *Streak*—fair, rarely continuous. "Smoke Gray."

A.m.—(1) very scant; (2) fair, confined to raised portions of colonies. (1) White; (2) "Smoke Gray." A.m. easily lost with continuous sub-culture.

Pigment—light golden brown after 2 weeks.

Egg albumen agar. (Fig. 7 A.)

Streak—(1) fair; (2) good, echinate. "Pale Smoke Gray." Margins crenate.

A.m.—fair. (1) White; (2) "Light Drab"; (3) "Smoke Gray" with white patches; (4) "Smoke Gray."

Guttation—copious, minute colourless drops over whole surface.

Pigment—after 3 weeks (1) "Ivory Yellow"; (2) "Cream."

Gelatine stab.

A.m.—abundant. White in centre; "Smoke Gray" on margin.

Liquefaction—napiform—10 mm. digested in 2 weeks; complete in 28 days.

Potato plug.

Growth—good, echinate, margins crenate.

A.m.—abundant. (1) "Pale Mouse Gray"; (2) "Hair Brown" dotted with white spots.

Colour of plug—(1) "Drab Gray"; (2) "Drab"; (3) "Fuscous Black."

Carrot plug.

Growth—(1) fair; (2) good, echinate. "Pale Smoke Gray."

A.m.—(1) scant; (2) good. (1) White; (2) "Pale Smoke Gray."

Colour of plug—"Benzo Brown."

Tyrosinase reaction—negative. Anaerobic growth—none.

Starch plate—positive—width of hydrolytic zone = 3 mm.

Nitrate reduction—positive 2—width of colour zone = 15 mm.

Growth at 37·5° C.—good.

Liquid Media.

Saccharose synthetic solution.

Growth—good; mainly discrete colonies on sides and bottom of tube. Colonies—white, dark spot in centre, later; disappearing. Surface growth—scant, discrete colonies.

A.m.—covers surface colonies. (1) "Light Vinaceous Fawn"; (2) "Ecru Drab."

Glycerine synthetic solution. (Fig. 7 B.)

Growth—good, discrete colonies, crystal-like, conical, clinging to sides and at bottom of tube. (1) Whitish; (2) "Pale Smoke Gray." Surface growth—colonies appearing after 3 weeks.

A.m.—scant. "Pale Smoke Gray."

Guttation—minute colourless drops.

Glucose broth.

Growth—(1) fair; (2) good. (1) on sides and base of tube; (2) all growth sinking to base forming sponge-like mass. Whitish. Surface growth—none.

Starch synthetic solution.

Growth—good; globular colonies mostly at bottom and on sides of flask. Surface growth—a few colonies.

A.m.—(1) white; (2) "Smoke Gray." *Guttation*—colourless drops.

Hydrolysis—positive, solution clear in 20 days.

Brom-cresol milk.

Growth—surface growth—good. "Hyssop Violet" to "Pearl Blue."

A.m.—none. *Coagulation*—positive.

Digestion of clot—after 3 weeks, 15 mm., finally 35 mm. Whey slightly cloudy.

Colour of whey—(1) "Dull Violet Blue"; (2) "Deep Hyssop Violet."

Strain 2 = *Actinomyces Setonii*.

Morphology.

Conidiophore. Much branched towards apex, lateral branches forking.

Conidia. Barrel-shaped, broader than long. 0·6 to 0·8 × 0·85μ. (Fig. 1.)

Cultural characteristics.

Solid Media.

Saccharose synthetic agar. (Fig. 5 A.)

Streak—(1) fair; (2) good, fine, smooth, soft; sending fine root-like projections into medium. Margins laciniate.

A.m.—abundant, early. "Olive Buff."

Pigment—"Cream" after 4 weeks.

Glycerine synthetic agar. (Fig. 5 B.)

Streak—good, crinkled at first. "Pale Smoke Gray." Edges of streak flat, crenate.

A.m.—abundant. (1) White; (2) "Pale Olive"; (3) "Olive Buff."

Guttation—minute colourless drops during first 10 days.

Pigment—(1) light golden brown; (2) "Colonial Buff"; (3) "Deep Colonial Buff."

Dextrose synthetic agar. (Fig. 6 A.)

Streak—good, echinate. "Pale Smoke Gray." Margins crenate.

A.m.—abundant. "Deep Olive Buff" with central streak of "Pale Olive Buff."

Guttation—minute drops, colourless.

Pigment—"Massicot Yellow."

Calcium malate glycerine agar.

Streak—good, echinate. "Pale Smoke Gray." Margins laciniate.

A.m.—abundant. "Ivory Yellow."

Pigment—(1) "Light Vinaceous Fawn"; (2) "Chamois."

Dextrose agar (Krainsky).

Streak—good, wrinkled. "Pale Smoke Gray." Margins minutely crenate.

A.m.—abundant. (1) White; (2) "Olive Buff."

Nutrient potato agar. (Fig. 6 C.)

Streak—rapid growth; good. Margins puckered.

A.m.—abundant, appearing with growth. Very smooth. White.

Guttation—during first 8 days minute amber coloured drops.

Pigment—golden brown.

Egg albumen agar. (Fig. 7 A.)

Streak—fairly good, flat, smooth. Border of growth slightly raised. Margins laciniate.

A.m.—abundant appearing with growth. (1) "Ivory Yellow" with patch of "Pale Greyish Vinaceous" at base; (2) "Ivory Yellow."

Pigment—after 4 weeks. "Cream."

Gelatin stab.

Growth. "Pale Smoke Gray"—good surface growth.

A.m.—covering surface growth. "White."

Liquefaction—napiform. 15 mm. digested in 2 weeks. Complete in 28 days.

Pigment—"Chamois."

Potato plug.

Growth—good, heavy wrinkled mass. Margins flat, crenate.

A.m.—abundant, rapid. (1) White; (2) "Tea Green"; (3) "Deep Olive Buff" with whitish edges.

Colour of plug—(1) "Light Drab"; (2) "Fuscous Black."

Carrot plug.

Growth—poor, semi-transparent.

A.m.—abundant. (1) White; (2) "Pale Smoke Gray."

Colour of plug. "Natal Brown" at base.

Tyrosinase reaction—negative. Anaerobic growth—none.

Starch plate—positive—width of hydrolytic zone = 3 mm.

Nitrate reduction—positive 3—width of colour zone = 15–20 mm.

Growth at 37·5° C.—good.

Liquid Media.

Saccharose synthetic solution.

Growth—good, smooth, soft, whitish flakes throughout medium, but collecting at base. Some surface growth.

A.m.—scant. (1) White; (2) "Pale Olive Buff."

Glycerine synthetic solution. (Fig. 7 B.)

Growth—good. (1) masses of soft white, downy flakes suspended in solution; (2) heavy surface growth, remainder at base, liquid quite clear.

A.m.—abundant. "Pale Olive Buff."

Guttation—large colourless drops on surface growth after 1 week.

Pigment—"Pale Turtle Green."

Glucose Broth.

Growth—(1) fair; (2) good. (1) White flaky masses confined to top and bottom of liquid; (2) after 3rd week, practically all growth on surface.

A.m.—abundant, wrinkled appearance. "Olive Buff."

Guttation—minute drops amber-coloured after 1 week.

Pigment—light golden brown.

Starch synthetic solution.

Growth—good, entirely confined to surface and bottom of medium. Surface layer, echinate.

A.m.—abundant—mealy. "Pale Olive Buff" spotted with white—these spots gradually disappearing.

Hydrolysis—complete in 1 week.

Pigment—"Straw Yellow" after 3 weeks.

Brom-cresol milk.

Growth—on surface—good. "Deep Delft Blue" in medium-heavy flocculent mass at base after 4 weeks.

A.m.—ring—white. *Coagulation*—positive.

Digestion of clot—20 mm. after 2 weeks. Final—complete.

Colour of digestion zone—(1) "Dull Dusky Purple"; (2) "Blackish Purple; (3) "Dark Maroon Purple."

Strain 3 = *Actinomyces marginatus.*

Morphology.

Conidiophore. Simple, branched.

Conidia. Spherical 0·87 × 0·8μ. (Fig. 1.)

Cultural characteristics.

Solid Media.

Saccharose synthetic agar. (Fig. 5 A.)

Streak—good, thin, spreading, finely echinate, sending fine root-like projections into medium. Margins slightly raised, finely crenate.

A.m.—abundant, appears with growth. "Olive Buff."

Pigment—"Cream colour" after 3 weeks.

Glycerine synthetic agar. (Fig. 5 B.)

Streak—good, thin, spreading, with raised border. Margins crenate.

A.m.—abundant, appears with growth. (1) "Pale Olive Buff"; (2) "Olive Buff."

Pigment—"Ivory Yellow."

Dextrose synthetic agar. (Fig. 6 A.)

Streak—fair, thin, flat, with distinct raised edge. "Pale Olive Gray."

A.m.—abundant. (1) "Pale Smoke Gray"; (2) "Olive Buff."

Pigment—"Cartridge Buff."

Calcium malate glycerine agar.

Streak—good, thin, slightly echinate. Slightly raised margin. "Dull Green Yellow."

A.m.—good. "Marguerite Yellow."

Guttation—small drops colourless, disappearing after 2nd week.

Pigment—"Marguerite Yellow."

Dextrose agar (Krainsky's).

Streak—fair, flat, with raised edges. Margin, crenate. Discrete colonies in the substratum. "Citrine Drab."

A.m.—(1) scant, confined to raised edges; (2) more abundant, gradually spreading. (1) White; (2) "Cartridge Buff."

Pigment—"Tilleul Buff" after 2 weeks.

Nutrient potato agar. (Fig. 6 C.)

Streak—heavy. "Smoke Gray." Thin ribbon-like edge.

A.m.—abundant. (1) White; (2) white tinged with yellow.

Pigment—(1) light golden brown; (2) deep golden brown.

Egg albumen agar. (Fig. 7 A.)

Streak—poor, thin, flat, transparent, more dense at bottom. "Pale Smoke Gray." Margins crenate. Some colonies in substratum.

A.m.—very scant, appears in 2nd week. White; a few spots of "Cartridge Buff" in 3rd week.

Pigment—(1) "Ivory Yellow"; (2) "Cream Buff"; (3) "Colonial Buff."

Gelatin stab.

Growth—"Pale Smoke Gray" with yellowish tinge.

A.m.—scant. White.

Liquefaction—stratiform. 15 mm. digested in 2 weeks. Complete in 4 weeks.

Pigment—"Marguerite Yellow" in 2nd week.

Potato plug.

Growth—good, raised, puckered, later echinate.

A.m.—abundant, appears with growth. (1) "Deep Colonial Buff" with white margins; (2) "Deep Olive Buff."

Colour of plug—(1) upper part "Deep Olive Gray," lower part "Puritan Gray"; (2) the whole (1) "Iron Gray," (2) "Fuscous Black."

Carrot plug.

Growth—poor, echinate. "Olive Buff."

A.m.—covers surface in first week. (1) White; (2) "Pale Smoke Gray."

Colour of plug—"Hair Brown," immediately below growth in 4th week.

Tyrosinase reaction—negative. "Light Grayish Olive" pigment after 4 weeks.

Starch plate—positive—width of hydrolytic zone = 4 mm.

Nitrate reduction—negative. Anaerobic growth—none.

Growth at 37·5° C.—good.

Liquid Media.

Saccharose synthetic solution.

Growth—fairly good—(1) in small flakes throughout medium; (2) a flaky deposit settles to bottom and star-like flakes remain suspended and clinging to sides of tube.

Glycerine synthetic solution. (Fig. 7 B.)

Growth—very poor, a few filmy flakes at base with scant surface growth, medium clear.

A.m.—very scant. "Pale Olive Buff."

Glucose broth.

Growth—good—(1) mostly at surface and on bottom in white spongy masses; (2) bottom growth gradually disappears leaving a few flakes; surface growth becomes abundant.

A.m.—(1) scant; (2) abundant. (1) White; (2) "Ivory Yellow."

Pigment—light golden brown.

Starch synthetic solution.

Growth—poor, flaky mass entirely at base. Solution nearly clear.

Pigment—straw colour after 4 weeks.

Hydrolysis—slow. Solution nearly clear in 20 days.

Brom-cresol milk.

Growth—surface—very good. "Deep Medici Blue." In medium—good, flocculent mass.

A.m.—white. *Coagulation*—positive.

Digestion of clot—20 mm. after 2 weeks. Final—complete.

Colour of digestion zone—(1) "Dull Dusky Purple"; (2) "Blackish Purple"; (3) "Dusky Auricula Purple."

Strains 4 and 12 = *Actinomyces praefecundus.*

Morphology.

Conidiophore. Branched—sporulation occurs first in the apical branches. Septa at base split.

Conidia. Barrel-shaped, broader than long. $0{\cdot}8 \times 0{\cdot}85\mu$. Germinating conidia in the spore chains often occur. (Figs. 1, 2.)

Cultural characteristics.

Solid Media.

Saccharose synthetic agar. (Fig. 5 A.)

Streak—good, thin, spreading. "Cream colour" on reverse side. Margins very laciniate.

A.m.—appears with growth. Very abundant. Fluffy. "Pale Olive Buff."

Pigment—"Cream Colour."

Glycerine synthetic agar. (Fig. 5 B.)

Streak—good, crinkled. "Pale Olive Gray." Margins crenate.

A.m.—abundant, smooth, white with patches of "Colonial Buff."

Guttation—large drops, colourless, mainly along border of streak; ceasing after 8 days.

Pigment—"Pale Chalcedony Yellow."

Dextrose synthetic agar. (Fig. 6 A.)

Streak—good, slightly echinate. "Pale Olive Gray." Margins (1) crenate; (2) markedly laciniate.

A.m.—very abundant, smooth. (1) White to "Pale Olive Buff"; (2) "Deep Olive Buff."

Guttation—colourless drops.

Pigment—(1) very light golden; (2) "Pinkish Buff"; (3) "Cartridge Buff."

Calcium malate glycerine agar.

Streak—good, slightly echinate. Margins crenate.

A.m.—abundant. (1) White; (2) "Pale Vinaceous Fawn."

Pigment—"Carnelian Red."

Dextrose agar (Krainsky's).

Streak—good, slightly echinate. "Pale Smoke Gray." Margins laciniate.

A.m.—abundant. "Pale Olive Buff."

Nutrient potato agar. (Fig. 6 C.)

Streak—good. (1) Wavy with crinkled edges; (2) centre of streak nearly flat. "Smoke Gray." Margins crenate.

A.m.—abundant, smooth. (1) White; (2) tinged with yellow.

Guttation—minute amber coloured drops mainly from border of growth, ceasing after 8 days.

Pigment—(1) light golden brown; (2) golden brown.

Egg albumen agar. (Fig. 7 A.)

Streak—good, thin, echinate. Margins laciniate.

A.m.—abundant. (1) "Ivory Yellow" but "Shell Pink Colour" at base; (2) "Ivory Yellow."

Pigment—(1) "Ivory Yellow"; (2) "Cartridge Buff."

Gelatine.

Growth—surface growth fairly good.

A.m.—white.

Liquefaction—napiform. 17 mm. digested in 14 days. Complete in 28 days.

Pigment—(1) "Onion-skin Pink"; (2) dark golden brown.

Potato plug.

Growth—good, wrinkled, echinate.

A.m.—abundant, appears with growth; (1) white at edges with yellowish tinge in centre; (2) "Pale Olive Buff."

Guttation—minute amber drops ceasing in 9 days, but later (after 20 days) some further drops much darker in colour may be exuded.

Colour of plug—(1) "Drab Gray"; (2) "Benzo Brown; (3) "Bone Brown."

Carrot plug.

Growth—poor. "Pale Smoke Gray."

A.m.—very scant. (1) White; (2) "Pale Smoke Gray."

Colour of plug—unchanged.

Tyrosinase reaction—negative. Anaerobic growth—none.

Starch plate—positive—width of hydrolytic zone = 5 mm.

Nitrate reduction—positive 3—colour diffused to edge of medium.

Growth at 37·5° C.—good.

Liquid Media.

Saccharose synthetic solution.

Growth—very good; (1) large white flakes at base and clinging to sides of tube (2) distributed throughout medium with good surface growth.

A.m.—(1) scant; (2) fair. (1) White; (2) "Tilleul Buff."

Glycerine synthetic solution. (Fig. 7 B.)

Growth—good; (1) large soft flakes throughout medium; (2) heavy surface growth, slight bottom growth, liquid clear. The surface growth later splits and curls up sides of tube like a skin.

A.m.—abundant, appearing in first week. (1) White; (2) "Pale Olive Buff."

Guttation—minute colourless drops ceasing after 3 weeks.

Pigment—none or a very light green after 4 weeks.

Glucose broth.

Growth—good in 14 hours—(1) whitish flaky masses throughout medium; (2) (after 1 week) rising entirely to surface, leaving medium clear. A slight growth at bottom of tube after 3 weeks. These gradations may repeat themselves.

A.m.—abundant, giving wrinkled appearance. "Pale Olive Buff."

Pigment—light golden brown.

Starch synthetic solution.

Growth—very good, confined to surface and bottom of flask. Flaky.

A.m.—abundant, white.

Pigment—"Ivory Yellow" after 4 weeks.

Hydrolysis—solution clear in 3 weeks.

Brom-cresol milk.

Growth—surface—good. "Deep Medici Blue." In medium, heavy, flocculent mass.

A.m.—scant, white.

Coagulation—positive.

Digestion of clot—10 to 15 mm. after 1 week. Final—complete.

Colour of digestion zone—(1) "Slate Purple"; (2) "Dull Dark Purple"; (3) "Blackish Purple"; (4) "Dusky Auricula Purple."

Strains 5 and 6 = *Actinomyces tenuis*.

orphology.

Conidiophore. Semi-prostrate, branching irregular. Septa split.

Conidia. Cylindrical, clearly articulated. 0·87 × 0·82 μ. (Fig. 1.)

Cultural characteristics.

Solid Media.

Saccharose synthetic agar. (Fig. 5 A.)

Streak—(1) fair, thin, flat, finely echinate. "Citrine Drab," margins crenate; (2) short fern-like projections. Good growth in substratum.

A.m.—appears with growth; good. "Deep Olive Buff."

Pigment—"Pale Orange Yellow."

Glycerine synthetic agar. (Fig. 5 B.)

Streak—good, thin, flat edges, slightly ridged with outer clear zone. Margins crenate.

A.m.—appears with growth; very good. (1) "Pale Olive"; (2) "Olive Buff."

Pigment—"Tilleul Buff."

Dextrose synthetic agar. (Fig. 6 A.)

Streak—(1) poor; (2) fair, spreading, thin, flat, finely echinate; raised edges.

A.m.—appears with growth; good. "Olive Buff."

Pigment—"Cream Colour."

Calcium malate glycerine agar.

Similar to Strain No. 3.

Dextrose agar (Krainsky's).

Streak—fair, flat, slightly echinate. "Citrine Drab." Margins crenate.

A.m.—abundant. (1) "Pale Olive Buff"; (2) "Cartridge Buff."

Pigment—"Ivory Yellow."

Nutrient potato agar. (Fig. 6 C.)

Streak—good, slightly wrinkled with flat borders. "Smoke Gray." Margins crenate.

A.m.—abundant. (1) White; (2) "Pale Vinaceous Fawn" with white edges.

Pigment—(1) golden brown; (2) deep golden brown.

Egg albumen agar. (Fig. 7 A.)

Streak—fair, flat, thin with raised edges. "Citrine Drab." Margins laciniate.

A.m.—fair. (1) White with borders of "Vinaceous Buff"; (2) "Ivory Yellow."

Pigment—"Ivory Yellow."

Gelatine.

Growth—"Pale Smoke Gray."

A.m.—rather scant. White.

Liquefaction—crateriform. 15 mm. digested in 14 days, complete in 28 days.

Pigment—"Marguerite Yellow."

Potato plug.

Growth—good, echinate. Margins crenate.

A.m.—abundant, appears with growth. "Deep Olive Buff" with slightly lighter edges.

Colour of plug—(1) "Puritan Gray"; (2) "Dark Olive; (3) "Fuscous Black."

Carrot plug.

Growth—fair, wrinkled. "Pale Smoke Gray"; echinate.

A.m.—abundant. (1) White; (2) "Pale Smoke Gray."

Colour of plug—unchanged.

Tyrosinase reaction—negative. Anaerobic growth—none.

Starch plate—positive. Width of hydrolytic zone = 5 mm. Width of incomplete zone = 10 mm.

Nitrate reduction—negative. Growth at 37·5° C.—good.

Liquid Media.

Saccharose synthetic solution.

Growth—poor, small white flakes distributed throughout medium and clinging to sides of tube; later, mostly falling to base. Slight surface growth.

A.m.—very scant. (1) "Pale Vinaceous Fawn"; (2) "Pale Olive Buff."

Glycerine synthetic solution. (Fig. 7 B.)

Growth—poor, soft, downy, white flakes mostly at base.

A.m.—very scant. "Olive Buff."

Glucose broth.

Growth—good after 14 hours. Surface and bottom growth; a few flakes suspended in medium. Whitish.

A.m.—abundant, wrinkled appearance. (1) "Olive Buff"; (2) "Deep Olive Buff."

Pigment—golden brown.

Starch synthetic solution.

Growth—fair, all at base.

Pigment—"Cartridge Buff" after 4th week.

Hydrolysis—complete in 15 days.

Brom-cresol milk.

Growth—surface—very good, "Deep Medici Blue." In medium—good.

A.m.—covering surface; "White."

Coagulation—positive.

Digestion of clot—15 mm. in 2 weeks. Final. Not quite complete.

Colour of digestion zone—(1) "Deep Slate Violet"; (2) "Dull Dusky Purple"; (3) "Blackish Purple"; (4) "Dark Maroon Purple."

Strain 7 = *Actinomyces gracilis.*

Morphology.

Conidiophore. Branching opposite. Sporulation eccentric.

Conidia. Oval or spherical. 0·8 to 0·87 × 0·87 μ. (Fig. 1.)

Cultural characteristics.

Solid Media.

Saccharose synthetic agar. (Fig. 5 A.)

Streak—(1) fair; (2) good, thin, flat; margins (1) feathery; (2) fantastic, fern-like outgrowths. "Pale Smoke Gray."

A.m.—(1) scant, confined to margin of streak, fluffy appearance; (2) fair. (1) "Smoke Gray"; (2) "Olive Buff."

Pigment—"Cream Colour."

Glycerine synthetic agar. (Fig. 5 B.)

Streak—good, slightly echinate; margins crenate.

A.m.—thin film; (1) white; (2) "Pale Olive Buff"; (3) "Cream Buff."

Pigment—(1) "Cream Buff"; (2) "Colonial Buff."

Dextrose synthetic agar. (Fig. 6 A.)

Streak—(1) fair; (2) good, smooth. "Pale Olive Gray"; margins crenate.

A.m.—abundant, white, smooth.

Guttation—colourless drops from pin-hole points.

Pigment—"Cream Colour."

Calcium malate glycerine agar.

Streak—good. (1) Thin, flat; (2) slightly echinate. Margins laciniate.

A.m.—abundant. "Cartridge Buff."

Pigment—(1) "Pale Cinnamon Pink"; (2) "Brazil Red."

Dextrose agar (Krainsky's).

Streak—fair, thin, flat, slightly echinate. "Pale Smoke Gray." Margins laciniate.

A.m.—abundant. "Pale Olive Buff."

Nutrient potato agar. (Fig. 6 C.)

Streak—good, very puckered with flat borders. "Vinaceous Buff." Margins slightly crenate.

A.m.—not abundant. "Pale Smoke Gray" with white edges.

Pigment—light golden brown.

Egg albumen agar. (Fig. 7 A.)

Streak—fair, thin, with raised edges. "Pale Smoke Gray." Margins laciniate.

A.m.—abundant. (1) White to "Olive Buff"; (2) "Olive Buff."

Pigment—"Ivory Yellow."

Gelatine.

Growth—"Pale Smoke Gray."

A.m.—covers surface growth. White.

Liquefaction—napiform. 13 mm. digested in 14 days. Complete in 26 days.

Pigment—(1) "Onion-skin Pink"; (2) light golden brown; (3) dark golden brown.

Potato plug.

Growth—good, echinate. Single colonies with very raised centres and slightly raised crenate margins.

A.m.—abundant, appears with growth. (1) "Pale Olive Gray"; (2) "Pale Olive Buff."

Colour of plug—(1) "Drab"; (2) "Bone Brown."

Carrot plug.

Growth—thin, spreading, slightly echinate. "Pale Smoke Gray."

A.m.—(1) scant; (2) abundant. (1) White; (2) "Drab Gray."

Colour of plug—"Bone Brown" in 4 weeks.

Tyrosinase reaction—negative. Anaerobic growth—none.

Starch plate—positive. Width of hydrolytic zone = 3–4 mm.

Nitrate reduction—positive, 1. Width of colour zone = 15 mm.

Growth at 37·5° C.—good.

Liquid Media.

Saccharose synthetic solution.

Growth—poor. Mainly at base and clinging to sides of tube. The medium becomes slightly milky.

A.m.—scant. (1) "Drab Gray"; (2) "Pale Smoke Gray."

Glycerine synthetic solution. (Fig. 7 B.)

Growth—very good; (1) soft, downy, white flakes throughout medium; (2) rises mainly to surface forming heavy growth, a small part sinking to bottom, a few flakes suspended in medium.

A.m.—abundant. (1) White; (2) "Pale Olive Buff."

Glucose broth.

Growth—good after 14 hours. (1) Confined to surface and bottom of medium; (2) bottom growth almost disappears, white flaky masses appear throughout medium.

A.m.—abundant, giving wrinkled appearance. "Pale Olive Buff."

Pigment—light golden brown after 3 weeks.

Starch synthetic solution.

Growth—very good, confined to surface and bottom of medium.

A.m.—abundant. (1) White; (2) "Ivory Yellow."

Pigment—"Straw Yellow" after 3 weeks.

Hydrolysis—complete in 7 days.

Brom-cresol milk.

Growth—surface, good. "Dark Gobelin Blue." In medium—good.

A.m.—ring and specks on surface. White.

Coagulation—positive.

Digestion of clot—28 mm. in 2 weeks. Final—complete.

Colour of digestion zone—(1) "Dull Dusky Purple"; (2) "Blackish Purple"; (3) "Dusky Auricula Purple."

Strain 8 = *Actinomyces Sampsonii.*

Morphology.

Conidiophore. Erect habit, sporulation regular.

Conidia. Cylindrical, varying greatly in length. 0·8 to 1 × 0·5μ. (Fig. 1.)

Cultural characteristics.

Solid Media.

Saccharose synthetic agar. (Fig. 5 A.)

Streak—(1) fair; (2) good, slightly rough. "Pale Smoke Gray" turning almost white; margins (1) crenate; (2) finely laciniate.

A.m.—very scant—appears in 3rd week at top of slope only. White.

Pigment—(1) "Cream"; (2) "Cartridge Buff."

Glycerine synthetic agar. (Fig. 5 B.)

Streak—(1) fair; (2) good, slightly echinate, almost white; margins crenate.

A.m.—fair, slow in appearing, white.

Pigment—"Cream Colour."

Dextrose synthetic agar. (Fig. 6 A.)

Streak—(1) poor; (2) fairly good, wrinkled. (1) White to "Pale Smoke Gray"; (2) "Pale Smoke Gray."

A.m.—scant, appears in 3rd week on upper part of streak only. White.

Pigment—"Pale Chalcedony Yellow."

Calcium malate glycerine agar.

Streak—fair, thin, flat, "Pale Smoke Gray." Margins (1) crenate; (2) laciniate.
A.m.—trace. White.

Dextrose agar (Krainsky's).

No growth.

Nutrient potato agar. (Fig. 6 C.)

Growth—(1) discrete colonies; (2) streak—good, smoothly wrinkled, spreading "Smoke Gray." Margins crenate.
A.m.—(1) scant, (2) abundant. White.
Pigment—(1) light golden brown; (2) golden brown.

Egg albumen agar. (Fig. 7 A.)

Streak—fair, thin, transparent. "Pale Smoke Gray." Margins crenate.
A.m.—very scant. White.
Pigment—"Marguerite Yellow" after 4 weeks.

Gelatine.

Growth—surface growth, scant. "Pale Smoke Gray."
A.m.—trace, whitish.
Liquefaction—crateriform. 15 mm. digested in 14 days. Complete in 28 days.

Potato plug.

Growth—no growth excepting in water.

Carrot plug.

Growth—very poor—one or two colonies, which shrivel and almost disappear.
A.m.—covers growth. White.
Colour of plug—unchanged.

Tyrosinase reaction—negative. A "Pale Pinkish Buff" pigment after 3 weeks.

Growth under anaerobic conditions—none. Starch plate—negative.

Nitrate reduction—positive 10. Colour diffused to edge of medium.

Growth at 37·5° C.—none.

Liquid Media.

Saccharose synthetic solution.

Growth—very poor, compact mass at base from which minute colonies radiate.

Glycerine synthetic solution. (Fig. 7 B.)

Growth—none.

Glucose broth.

Growth—(1) good, colonies coalescing at bottom with a few clinging to sides of tube; (2) good bottom growth—white spongy masses, a few colonies floating in medium and on surface; (3) abundant bottom growth—large sponge-like colonies, large number of colonies clinging to sides of tube.
A.m.—white.

Starch synthetic solution.

Growth—very slight.
A.m.—none.
Hydrolysis—none.

Brom-cresol milk.

Growth—surface—thin ring after 2 weeks. "Light Medici Blue." In medium—numerous discrete compact colonies clinging to sides of tube and suspended throughout medium. Whitish.

A.m.—none.

Clotting—none.

Hydrolysis—none.

Colour of medium—no change.

Strain 9 = *Actinomyces viridis*.

Morphology.

Conidiophore. Branching irregular, the uppermost branches sometimes showing a whip-like appearance. Sporulation regular. Septa, split.

Conidia. Cylindrical. 0·85 to 0·88 × ·08 μ. (Fig. 1.)

Cultural characteristics.

Solid Media.

Saccharose synthetic agar. (Fig. 5 A.)

Streak—(1) fair; (2) good, thin, flat, distinct raised frill-like crenate margins.

A.m.—appears with growth. Good. "Deep Grayish Olive."

Pigment—(1) "Pale Green Yellow"; (2) "Dull Blackish Green" immediately below growth.

Glycerine synthetic agar. (Fig. 5 B.)

Streak—(1) poor; (2) spreading over whole surface; thin, flat. (1) "Grayish Olive"; (2) "Natal Brown"; (3) "Benzo Brown." Margins crenate.

A.m.—scant. (1) White; (2) "Pale Olive Buff"; (3) "Pale Smoke Gray."

Pigment—(1) "Tilleul Buff"; (2) "Vinaceous Buff."

Dextrose synthetic agar. (Fig. 6 A.)

Streak—good, smooth, raised. "Olive Buff." Margins flat, slightly crenate.

A.m.—abundant. (1) "Light Olive Gray"; (2) "Deep Mouse Gray."

Guttation—minute drops mainly along sides of growth. (1) Colourless; (2) amber.

Pigment—(1) "Light Chalcedony Yellow"; (2) "Citron Green"; (3) "Dull Green Yellow"; (4) "Cerro-Green." Immediately below growth "Dull Greenish Black."

Calcium malate glycerine agar.

Streak—good, echinate. (1) "Elm Green"; (2) almost black. Margins laciniate.

A.m.—abundant. "Light to Deep Grayish Olive."

Guttation—after 3 weeks. Minute colourless drops.

Pigment—"Elm Green."

Dextrose agar (Krainsky's).

Streak—good, raised, wrinkled. "Citrine Drab." Margins laciniate. Discrete colonies in substratum.

A.m.—fair. (1) "Hair Brown"; (2) "Deep Olive Gray"; (3) "Dark Olive Gray."

Pigment—(1) "Ivory Yellow"; (2) "Pale Pinkish Buff."

Nutrient potato agar. (Fig. 6 C.)

Streak—good, slightly wrinkled. "Smoke Gray." Margins crenate.

A.m.—very scant. White.

Pigment—(1) light golden brown; (2) golden brown.

Egg albumen agar. (Fig. 7 A.)

Streak—(1) fair; (2) good, thin, echinate, raised edges. Margins crenate.

A.m.—abundant. "Deep Olive."

Pigment—(1) "Pale Veronese Green"; (2) "Civette Green"; (3) "Dark Yellowish Green."

Gelatine.

Growth—"Pale Smoke Gray."

A.m.—scant. (1) White; (2) "Smoke Gray."

Guttation—minute drops.

Liquefaction—crateriform. 15 mm. digested in 14 days. Complete in 28 days.

Pigment—light golden brown.

Potato plug.

Growth—(1) poor; (2) good, echinate. (1) "Iron Gray"; (2) "Deep Olive Gray" (3) "Straw Yellow"; (4) "Saccardo's Olive"; plug becomes flabby.

A.m.—none.

Colour of plug—(1) "Pallid Mouse Gray"; (2) "Bone Brown."

Carrot plug.

Growth—good, thin, spreading, echinate. (1) "Dark Olive Gray"; (2) "Iron Gray."

A.m.—scant, appears after 7 days. White.

Colour of plug—"Bone Brown."

Tyrosinase reaction—negative. Anaerobic growth—none.

Starch plate—positive. Width of hydrolytic zone = 5 mm.

Nitrate reduction—positive 9. Width of colour zone = 30 mm.

Growth at 37·5° C.—good.

Liquid Media.

Saccharose synthetic solution.

Growth—good; large flaky masses throughout medium, which, later, mainly rise to surface.

A.m.—abundant. (1) "Grayish Olive"; (2) "Pale Olive Gray"; (3) "Pale Smoke Gray."

Pigment—"Pale Grass Green."

Glycerine synthetic solution. (Fig. 7 B.)

Growth—good; (1) soft, downy flakes confined to lower half of tube and large number minute specks on sides of tube which persist, not shown in photograph; (2) fair surface growth. (1) White; (2) "Light Vinaceous Fawn."

A.m.—fair. (1) "Pale Smoke Gray"; (2) "Drab Gray."

Glucose broth.

Growth—good; on surface—fair, flaky, adhering to tube. At base—good, flaky; medium clear.

A.m.—(1) fair; (2) good. (1) "Smoke Gray"; (2) "Deep Mouse Gray."

Pigment—(1) "Light Vinaceous Cinnamon"; (2) "Vinaceous Rufous" deepening.

Starch synthetic solution.

Growth—very good, entirely surface and bottom.

A.m.—abundant. (1) White; (2) "Mouse Gray."

Pigment—(1) "Light Corinthian Red"; (2) "Pinkish Buff."
Hydrolysis—solution clear in 7 days.

Brom-cresol milk.
Growth—surface, good. "Deep Glaucous Gray." In medium—cloudy growth.
A.m.—scant. White.
Coagulation—positive after 3 weeks.
Digestion of clot—slow. 40 mm. after 3 weeks. Final, complete.
Colour of digestion zone—(1) "Dull Blue Violet"; (2) "Cotinga Purple."

Strain 10 = *Actinomyces Loidensis*.

Morphology.
Conidiophore. Erect. Sporulation irregular.
Conidia. Cylindrical. 0·87–0·98 × 0·87–0·95 μ. (Fig. 1.)

Cultural characteristics.

Solid Media.

Saccharose synthetic agar. (Fig. 5 A.)
Streak—(1) poor; (2) fair, thin, flat, spreading, finely echinate. (1) "Pale Smoke Gray"; (2) "Light Yellowish Olive." Margins crenate.
A.m.—scant. "Deep Olive Buff."
Pigment—(1) "Sulphur Yellow"; (2) "Maize Yellow."

Glycerine synthetic agar. (Fig. 5 B.)
Streak—fair, thin, finely echinate, flat, spreading. "Tea Green." Margins crenate.
A.m.—(1) scant; (2) thin film. (1) White; (2) "Pale Olive Buff."
Pigment—"Pale Chalcedony Yellow."

Dextrose synthetic agar. (Fig. 6 A.)
Streak—(1) poor; (2) fair, spreading, thin, slightly echinate; "Grayish Olive." Margins crenate.
A.m.—not abundant. (1) "Deep Olive Buff"; (2) the same with patch of "Light Vinaceous Fawn."
Pigment—(1) very light golden; (2) "Ivory Yellow."

Calcium malate glycerine agar.
Streak—good, slightly echinate. "Pale Smoke Gray." Margins laciniate.
A.m.—abundant. "Cartridge Buff."
Pigment—after 4 weeks a trace. "Sea Foam Green."

Dextrose agar (Krainsky's).
Streak—fair, very fine, becoming slightly raised and echinate. "Grayish Olive." Margins crenate.
A.m.—abundant. (1) "Olive Buff"; (2) "Cartridge Buff."
Pigment—"Ivory Yellow."

Nutrient potato agar. (Fig. 6 C.)
Streak—good, wavy, echinate. "Smoke Gray." Margins crenate.
A.m.—abundant. "Pale Olive Buff."
Pigment—(1) golden brown; (2) deep golden brown. Black immediately beneath growth.

Egg albumen agar. (Fig. 7 A.)

Streak—fair, thin, flat, raised edges. "Citrine Drab." Margins laciniate.

A.m.—scant. (1) "Ivory Yellow"; (2) "Cartridge Buff."

Pigment—"Light Corinthian Red."

Gelatine.

Growth—surface growth puckered, flakes of growth throughout liquefied portion. "Pale Smoke Gray."

A.m.—(1) Scant; (2) thin layer. White.

Liquefaction—crateriform. 17 mm. digested in 14 days. Complete in 28 days.

Pigment—"Marguerite Yellow."

Potato plug.

Growth—good, echinate.

A.m.—abundant, appears with growth. (1) "Light Olive Gray," edges white; (2) "Deep Olive Buff."

Colour of plug—(1) "Light Olive Gray"; (2) "Grayish Olive"; (3) "Bone Brown."

Carrot plug.

Growth—good, slightly echinate. "Pale Smoke Gray."

A.m.—abundant. (1) White with yellowish tinge; (2) "Light Olive Gray"; (3) "Pale Olive Buff."

Colour of plug—"Clove Brown" under and around growth.

Tyrosinase reaction—negative. Anaerobic growth—none.

Starch plate—positive. Width of hydrolytic zone 4–5 mm.

Nitrate reduction—negative. Growth at 37·5° C.—good.

Liquid Media.

Saccharose synthetic solution.

Growth—poor; (1) a few small white flakes throughout medium; (2) a few appear on surface and remainder sink to bottom—liquid clear.

A.m.—scant. (1) White; (2) "Pale Olive Buff."

Glycerine synthetic solution. (Fig. 7 B.)

Growth—poor, small white flakes, mostly at bottom, a few on surface.

A.m.—very scant. "Tilleul Buff."

Glucose broth.

Growth—good; (1) mostly on surface and at bottom with few flakes suspended; (2) surface growth abundant; bottom growth fair—flaky masses. (1) "Pale Smoke Gray"; (2) "Golden Brown."

A.m.—fair. "Olive Buff."

Pigment—(1) golden brown; (2) deep golden brown.

Starch synthetic solution.

Growth—fair, entirely at bottom of flask.

Pigment—"Ivory Yellow" made 4 weeks.

Hydrolysis—solution clear in 20 days.

Brom-cresol milk.

Growth—on surface—very good. "Deep Medici Blue"; in medium—flocculent mass.

A.m.—good. White.

Coagulation—positive.

Digestion of clot—20 mm. after 2 weeks. Final—nearly complete.

Colour of digestion zone—(1) "Dull Dark Purple"; (2) "Dark Hyssop Violet"; (3) "Dusky Auricula Purple."

STRAIN 11 = *Actinomyces spiralis.*

MORPHOLOGY.

Conidiophore. Branching simple—terminal branches closely spiral and densely granular. Sporulation—confined to the spiral filaments. Septa—split.

Conidia. Cylindrical. 1 to $1{\cdot}7\mu \times 0{\cdot}87\mu$. (Fig. 2.)

CULTURAL CHARACTERISTICS.

SOLID MEDIA.

SACCHAROSE SYNTHETIC AGAR. (Fig. 5 A.)

Colonies—small, umbonate, forming roughly echinate or granular growth. *Streak*—(1) very poor; (2) good. Margins ragged.

A.m.—(1) very scant; (2) abundant. Shows 3 colours in different parts of streak at same time. White; "Vinaceous Buff"; "Dark Grayish Olive."

Guttation—minute drops on a few colonies.

Pigment—"Pale Vinaceous Fawn."

GLYCERINE SYNTHETIC AGAR. (Fig. 5 B.)

Colonies—small, flat, forming rough granular surface. *Streak*—(1) poor; (2) good. "Pale Smoke Gray."

A.m.—good, slow in appearing. (1) White; (2) "Tilleul Buff."

Guttation—colourless drops.

Pigment—(1) "Tilleul Buff"; (2) "Vinaceous Buff."

DEXTROSE SYNTHETIC AGAR. (Fig. 6 A.)

Colonies—small, umbonate, forming rough echinate or granular surface. *Streak*—(1) poor; (2) fair. "Pale Smoke Gray."

A.m.—(1) scant; (2) fair. White.

Guttation—clear amber drops.

Pigment—(1) "Ivory Yellow"; (2) "Buff Pink."

CALCIUM MALATE GLYCERINE AGAR.

Growth—trace after 3 weeks; few minute colonies, entire, smooth. "Pale Smoke Gray."

A.m.—on colonies at top end of slope. White.

DEXTROSE AGAR (Krainsky's).

No growth.

NUTRIENT POTATO AGAR. (Fig. 6 C.)

Streak—fair, echinate, granular. "Pale Smoke Gray."

A.m.—abundant. (1) White; (2) "Pale Olive Buff."

Pigment—light golden brown.

EGG ALBUMEN AGAR. (Fig. 7 A.)

Growth—none until 2nd week; (1) small colonies; (2) poor, thin, spreading streak. "Pale Smoke Gray."

A.m.—none.

Pigment—"Marguerite Yellow" after 4 weeks.

Gelatine.

Growth—surface scant. "Pale Smoke Gray."
A.m.—scant. White.
Liquefaction—napiform. 10 mm. digested in 14 days. Complete in 28 days.

Potato plug.

Growth—poor, wrinkled, single colonies markedly umbonate, very puckered. "Pale Grayish Vinaceous."
A.m.—(1) scant; (2) good. (1) White; (2) "Pale Grayish Vinaceous."
Colour of Plug—"Wood Brown" around and below growth.

Carrot plug.

Growth—very poor.
A.m.—trace. White.
Colour of plug—unchanged.

Tyrosinase reaction—negative. Anaerobic growth—none.
Starch plate—negative. Nitrate reduction—negative.
Growth at 37·5° C.—none.

Liquid Media.

Saccharose synthetic solution.

Growth—none.

Glycerine synthetic solution.

Growth—none.

Glucose broth.

Growth—(1) poor; (at bottom of tube), minute, discrete colonies; (2) a few colonies on surface; (3) bottom growth, compact, spongy, heaped up colonies.
A.m.—scant, appears after 3 weeks. White.

Starch synthetic solution.

Growth—very slight.
A.m.—none.
Hydrolysis—none.

Brom-cresol milk.

Growth—surface—very good. "Olive Gray"; in medium—cloudy mass.
A.m.—abundant. White.
Coagulation—positive.
Digestion of clot—20 mm. after 2 weeks. Final—complete.
Colour of digestion zone—final "Cotinga Purple."

Strain 13 = *Actinomyces maculatus.*

Morphology.

Vegetative hyphae, extremely minute.
No aerial mycelium or only trace on any media.
Colonies of spores forming short chains appear as blackish green centres to the colonies on nearly all media—possibly Chlamydospores or very simple conidiophores.
Spores—from 1-month-old egg albumen agar and saccharose synthetic agar cultures—spherical. $0{\cdot}54\mu \times 0{\cdot}6\mu$.

Cultural characteristics. (*A.m.*—is not noted unless produced.)

Solid Media.

Saccharose synthetic agar. (Fig. 5 A.)

Colonies—round, flat, slightly raised centres.

Streak—(1) very poor, consisting of minute colonies which slowly coalesce; (2) fair growth. (1) "Pale Smoke Gray"; (2) "Light Pinkish Cinnamon." After the 3rd week numerous colonies show "Dull Greenish Black" centres.

Glycerine synthetic agar.

Growth—poor, consisting at first of very small discrete colonies. (1) "Shell Pink"; (2) "Grayish Olive."

Dextrose synthetic agar.

Streak—good, thin, flat, spreading, almost black with greenish tinge. Margins crenate.

Calcium malate glycerine agar. (Fig. 6 B.)

Streak—fairly good; thin, flat, spreading, biting into medium. "Pale Smoke Gray." Later, some colonies show "Dull Blackish Green" centres.

A.m.—trace appears on dried part of slope in 4th week. White.

Dextrose agar (Krainsky's).

Colonies—numerous, minute, slightly raised centres; later dark central spot.

Streak—fair, thin, flat, spreading. "Onion-skin Pink."

Nutrient potato agar.

Streak—fair, echinate. "Vinaceous-Tawny."

Pigment—"Vinaceous-Tawny."

Egg albumen agar. (Fig. 7 A.)

Streak—(1) fairly good; (2) good. Very thin, flat. (1) "Pale Smoke Gray"; (2) "Pale Ochraceous Salmon." Margins crenate. Minute blackish points arise scattered over the streak marking the centres of the original colonies.

Gelatine.

Growth—poor.

Liquefaction—napiform; slow; complete in 6 weeks.

Potato plug.

Growth—restricted, echinate, very raised. "Onion-skin Pink."

A.m.—scant, appearing in 4th week. White.

Colour of plug—(1) "Drab Gray"; (2) "Bone Brown."

Carrot plug.

Growth—very poor, few colonies only, transparent, jelly-like, gradually becoming shrivelled.

Colour of plug—unchanged.

Tyrosinase reaction—negative.

Anaerobic growth—appeared in first week, ultimately forming a thin, flat, transparent streak.

Starch plate—positive. Width of hydrolytic zone=4–5 mm. Width of incomplete ditto=8 mm.

Nitrate reduction—negative. Growth at 37·5° C.—good.

Liquid Media.

Saccharose synthetic solution.

Growth—very poor at bottom only, few small flakes which coalesce to thick skin-like piece. "Light Vinaceous Fawn."

Glycerine synthetic solution. (Fig. 7 B.)

Growth—poor, at bottom only; (1) numerous minute colonies; (2) fine flakes.

Glucose broth.

Growth—fair at bottom, numerous minute colonies. Whitish.

Starch synthetic solution.

Growth—surface—none.

In medium—(1) innumerable minute colonies in suspension with a few clinging to the sides of the flask; (2) colonies sink to bottom; with the precipitated starch, a softly flocculent mass is formed. The colonies show greenish black centres.

Hydrolysis—supernatant liquid clear.

Brom-cresol milk.

Growth—very slight, almost entirely at bottom, where some colonies are "Apricot Buff."

Coagulation—none.

Colour of milk—unchanged.

Strain 14 = *Actinomyces salmonicolor*.

Morphology.

Vegetative hyphae extremely minute. Forms no aerial mycelium or only trace on any media.

Colonies of spores forming short chains appear as darker centres to the colonies on certain media—as in Strain 13.

Conidia—from 1-month-old egg albumen agar and saccharose synthetic agar cultures—spherical or barrel-shaped. $0{\cdot}5$ to $0{\cdot}8 \times 0{\cdot}4$ to $0{\cdot}8\mu$.

Cultural characteristics. (*A.m.*—is not noted unless produced.)

Solid Media.

Saccharose synthetic agar. (Fig. 5 A.)

Colonies—round, slightly raised centres.

Streak—(1) very poor, minute colonies; (2) fairly good after 4 weeks. (1) "Pale Smoke Gray"; (2) "Light Pinkish Cinnamon."

Glycerine synthetic agar.

Streak—poor, thin, flat, spreading, almost transparent. (1) "Pale Smoke Gray"; (2) "Dark Grayish Olive."

Dextrose synthetic agar.

Colonies—small, "Pale Smoke Gray" with "Ochre Red" centres, slightly raised, growing 1 mm. into medium.

Streak—fair, spreading. "Dark Livid Purple."

Pigment—slight golden tinge.

Calcium malate glycerine agar. (Fig. 6 B.)

Streak—fair, thin, flat, spreading, biting into medium. "Pale Smoke Gray." Very like No. 13, but colonies do not show coloured centres.

A.m.—trace on dried part of slope after 4th week. White.

Dextrose agar (Krainsky's).

Colonies—few, minute, then raised, not coalescing. (1) "Pinkish Buff"; (2) "Buffy Brown"; (3) after 8 weeks, almost black.

Nutrient potato agar.

Colonies—few, slightly raised, wrinkled; later, acervulate. (1) Pinkish; (2) "Burnt Umber" with pinkish edges.

Egg albumen agar. (Fig. 7 A.)

Colonies—small, entire, flat, growing into medium.

Streak—(1) poor; (2) fairly good. (1) "Pale Ochraceous Salmon"; (2) "Pale Ochraceous Buff." A few colonies show minute blackish centres.

Gelatine.

Growth—poor.

Liquefaction—napiform. Slow. Complete in 34 days.

Potato plug.

Growth—restricted, raised, wrinkled. (1) "Ochre Red"; (2) "Dark Livid Brown."

Colour of plug—"Drab Gray."

Carrot plug.

No growth.

Tyrosinase reaction—negative. Anaerobic growth—none.

Growth at 37·5° C.—good.

Starch plate—positive. Width of hydrolytic zone = 1–2 mm.

Nitrate reduction—positive 1. Colour diffused to edge of medium.

Liquid Media.

Saccharose synthetic solution.

Growth—very poor, at base only. (1) A slight cloudy mass becoming skin-like; (2) compactly sponge-like.

Glycerine synthetic solution. (Fig. 7 B.)

Growth—poor. Flaky, at base, only.

Glucose broth.

Growth—fair, at base; (1) in compact skin-like pieces; (2) sponge-like mass.

Starch synthetic solution.

Growth—on surface none; in medium (1) innumerable minute colonies in suspension and clinging to the sides of the flask; (2) colonies, large, round, forming a thick layer at the bottom of the flask resembling tapioca. Some show "Vinaceous Buff" centres; those on the sides have fringed edges and if left above the surface of the medium become a "Deep Livid Brown."

Hydrolysis—supernatant liquid slightly cloudy.

Brom-cresol milk.

Growth—on surface, fair, ring of colonies. "Ochraceous Salmon"; in medium—numerous colonies clinging to sides of tube. "Pale Ochraceous Salmon" centres, with lighter margins.

Coagulation—positive after 3 weeks—a very thick solid curd separates out.

Digestion—after 4 weeks—average depth 20 mm.; clot remains intact, no zone formation.

Colour changes—curd. (1) "Puritan Gray"; (2) "Court Gray"; (3) "Light Mineral Gray." Whey—"Purple Drab."

Strain 15 = *Actinomyces Wedmorensis.*

Morphology (from saccharose synthetic agar culture).

Conidiophore. Simple, branched, closely septate; sporulation regular.

Conidia. Oval to cylindrical. 0·8μ to 0·85$\mu \times$ 0·6 to 0·75μ. (Fig. 2.)

Cultural characteristics.

Solid Media.

Saccharose synthetic agar. (Fig. 5 A.)

Streak—(1) fairly good, flat, thin fine; (2) grows deeply into substratum. "Pale Smoke Gray"; margins laciniate.

A.m.—thin; (1) white; (2) "Light Mouse Gray"; (3) "Pale Drab Gray."

Glycerine synthetic agar.

Streak—good, raised, echinate; "Pale Smoke Gray." Margins crenate.

A.m.—covers surface. White.

Dextrose synthetic agar.

Streak—very good. "Pale Smoke Gray" with crater-like dark spots.

A.m.—fair. "Pale Smoke Gray."

Calcium malate glycerine agar. (Fig. 6 B.)

Streak—good, flat, biting into medium. Margin crenate. Colonies retain their shape in the streak; (1) centres "Light Ochraceous Buff," then black; (2) colonies "Deep Grayish Olive" to "Deep Olive Buff"; (3) "Dark Olive."

A.m.—trace after 3 weeks. "7 Deep Gull Gray."

Pigment—(1) "Cream Colour"; (2) "Deep Olive Buff."

Dextrose agar (Krainsky's).

Streak—good, raised, echinate. "Pale Smoke Gray." Margins laciniate.

A.m.—covering growth. (1) "Smoke Gray"; (2) "Hair Brown."

Nutrient potato agar.

Streak—good, wrinkled, with loose skin-like appearance. "Pale Smoke Gray." Margins (1) crenate; (2) laciniate.

A.m.—none.

Egg albumen agar. (Fig. 7 A.)

Streak—good, thin, flat, spreading, biting into medium. "Pale Smoke Gray." Margins crenate.

A.m.—(1) scant; (2) fairly good. "Smoke Gray."

Gelatine.

Growth—fair.

A.m.—none.

Liquefaction—napiform. Complete in 24 days.

Potato plug.

Growth—good, raised, wrinkled. "Smoke Gray."

A.m.—covering growth. White.

Colour of plug—"Drab" deepening.

Carrot plug.

Growth—few colonies characteristically flat and rosette-like. (1) "Pale Smoke Gray"; (2) "Olive Buff."

A.m.—thin; on margins only. (1) White; (2) "Pale Olive Gray."

Colour of plug—unchanged.

Tyrosinase reaction—negative. Anaerobic growth—none.

Starch plate—positive. Width of hydrolytic zone = 4 mm. Width of incomplete zone = 6 mm.

Nitrate reduction—positive 3. Width of colour zone = 10 mm.

Growth at 37·5° C.—good.

Liquid Media.

Saccharose synthetic solution.

Growth—poor; (1) flaky mass at base, with a few minute flakes clinging to side of tube; (2) flaky bottom growth only.

Glycerine synthetic solution. (Fig. 7 B.)

Growth—good; (1) soft spongy mass at base with innumerable minute colonies on sides of tube; (2) numbers of colonies throughout the medium, with fair surface growth; (3) most of the surface growth sinks to the bottom, granular.

A.m.—appears during surface growth. "Pale Mouse Gray" flecked with white.

Glucose broth.

Growth—fairly good. (1) Masses of small filmy flakes with numerous minute colonies at base of tube; (2) slight surface growth. Whitish.

A.m.—none.

Starch synthetic solution.

Growth—on surface—a few colonies and rim after 3 weeks. White; at base—(1) heavy flocculent growth; (2) soft, flaky mass.

A.m.—fair. "Pale Smoke Gray."

Hydrolysis—supernatant liquid clear.

Pigment—"Pale Chalcedony Yellow."

Brom-cresol milk.

Growth—on surface—fair; "Vetiver Green"; in medium—none.

A.m.—none.

Coagulation—positive—similar to Strain 14.

Colour changes—curd—(1) "Gnaphalium Green"; (2) "Mineral Gray." Whey—"Purple Drab."

Strain 16 = *Actinomyces coroniformis.*

Morphology—on egg albumen agar culture—3 weeks old.

Conidiophore. Simple, branched. Septa split. Sporulation regular.

Conidia—oval. $0{\cdot}8 \times 0{\cdot}6\mu$. (Fig. 2.)

Cultural characteristics.

Solid Media.

Saccharose synthetic agar. (Fig. 5 A.)

Colonies—round, flat.

Streak—(1) poor; (2) fair; small discrete colonies, which partially coalesce. (1) "Pale Smoke Gray"; (2) "Vetiver Green."
A.m.—on edges of colonies only. White.

Glycerine synthetic agar.
Colonies—small, raised centres.
Streak—poor.
A.m.—covering colonies. White.

Dextrose synthetic agar.
Streak—poor, small discrete colonies, slightly raised, growing 1 mm. into medium. "Pale Smoke Gray."
A.m.—none.

Calcium malate glycerine agar. (Fig. 6 B.)
Colonies—small, round, slightly umbonate, growing well into medium, in compact, inverted hemispherical masses.
Streak—poor, discontinuous. "Pale Smoke Gray."
A.m.—(1) scant, rimming colonies; (2) closing over the colonies leaving pinpoint crater-like depression in entire. White.
Pigment—"Ivory Yellow" in 4th week.

Dextrose agar (Krainsky's).
Growth—very slight. "Pale Smoke Gray."
A.m.—none.

Nutrient potato agar.
Streak—fair, acervate, wrinkled; single colonies show raised centres. "Pale Smoke Gray."
A.m.—none.

Egg albumen agar. (Fig. 7 A.)
Streak—fairly good, discrete colonies, flat, slightly raised centres, growing into medium. "Pale Smoke Gray," larger colonies "Vinaceous Fawn."
A.m.—scant, rimming each colony, closing over the colonies leaving crater-like depression in centre. White.

Gelatine.
Growth—fair.
A.m.—none.
Liquefaction—napiform. Fair. Complete in 28 days.

Potato plug.
Growth—fair, raised, echinate. "Drab Gray."
A.m.—fair. White.
Colour of plug—"Hair Brown" under and around growth.

Carrot plug.
No growth.

Tyrosinase reaction—negative. Anaerobic growth—none.

Starch plate—negative.

Nitrate reduction—positive 7. Width of colour zone = 8 mm.

Growth at 37·5° C.—fair.

Liquid Media.

Saccharose synthetic solution.

Growth—poor; at base only. (1) Minute flakes which increase; (2) compact blobs.

Glycerine synthetic solution. (Fig. 7 B.)

Growth—very poor, few small flakes at base.

Glucose broth.

Growth—fair, at base only; (1) discrete colonies; (2) compact masses.

Starch synthetic solution.

Growth—on surface—none. In medium—(1) large numbers of small colonies in suspension; (2) colonies sink and with the precipitated starch form softly flocculent mass.

Hydrolysis—supernatant liquid slightly cloudy.

Brom-cresol milk.

Growth—a few colonies at surface.

A.m.—none. *Coagulation*—none. *Colour change*—none.

Strain 17 = *Actinomyces scabies* (Thaxter) Güssow emend. Millard and Burr.

Morphology—on egg albumen agar culture—24 days old.

Conidiophore. Branched, terminal branches long and sometimes spirally coiled. Septa of "attenuated isthmus" type.

Conidia. Barrel-shaped. 0·8 to 1·7μ × 0·5 to 0·8μ. (Fig. 2.)

Cultural characteristics.

Solid Media.

Saccharose synthetic agar. (Fig. 5 A.)

Colonies—large, umbonate.

Streak—very good; (1) crossed in every direction by the junction lines of coalescing colonies; (2) heavy, wrinkled, nodulose. (1) "Pale Smoke Gray"; (2) "Smoke Gray."

A.m.—(1) scant; (2) abundant. (1) White; (2) "Pale Neutral Gray" on upper part of streak; (3) "Pale Smoke Gray."

Guttation—minute colourless drops in 4 weeks.

Pigment—(1) "Marguerite Yellow"; (2) "Colonial Buff."

Glycerine synthetic agar. (Fig. 5 B.)

Colonies—medium-sized umbonate.

Streak—good, very uneven, nodulose. "Pale Smoke Gray." Margins slightly fringed with fine growth.

A.m.—(1) scant; (2) abundant. (1) White; (2) white sprinkled with "Pale Mouse Gray."

Guttation—minute, colourless drops after 4 weeks.

Pigment—"Ivory Yellow."

Dextrose synthetic agar. (Fig. 5 C.)

Streak—(1) fair (numerous discrete colonies); (2) good, nodular. (1) "Pale Smoke Gray"; (2) same with streak of "Chamois" down the centre; (3) all "Chamois."

A.m.—(1) scant, scattered; white; (2) good; "Pale Gull Gray."

Pigment—(1) "Cream Buff"; (2) "Chamois."

Calcium malate glycerine agar. (Fig. 6 B.)

Colonies—large, flatly conical, growing deeply into medium in spreading filmy masses.

Streak—(1) good, not continuous; (2) margins markedly laciniate finally linking up the colonies. (1) "Pale Smoke Gray" with dirty yellow centres; (2) "Chamois"; (3) "Honey Yellow"; (4) "Buckthorn Brown."

A.m.—scant. White.

Pigment—"Ivory Yellow."

Dextrose agar (Krainsky's).

Colonies—large, slightly raised, very definite flat margins.

Streak—good, very irregular in outline with characteristic filmy masses growing deeply into substratum. "Pale Smoke Gray."

A.m.—(1) scant; (2) good. (1) White; (2) "Pale Smoke Gray"; (3) "Smoke Gray."

Guttation—fair, minute colourless drops.

Pigment—(1) "Naphthalene Yellow"; (2) "Marguerite Yellow."

Nutrient potato agar. (Fig. 6 C.)

Colonies—medium size, umbonate, lustrous.

Streak—(1) fair; (2) good, nodulose, acervate. "Pale Smoke Gray."

A.m.—none.

Pigment—deep golden brown.

Egg albumen agar. (Fig. 7 A.)

Streak—very good, formed from large, flat, smooth, spreading colonies. Each colony has small central pimple on which aerial mycelium first appears. Grows deeply into medium in filmy spreading masses. "Pale Smoke Gray." Margins (1) lobular; (2) laciniate.

A.m.—abundant. "Pale Smoke Gray."

Guttation—minute colourless drops in 3rd week.

Pigment—"Naphthalene Yellow."

Gelatine.

Growth—beaded. (1) Very poor; (2) fair.

A.m.—slight. White.

Liquefaction—napiform. 10 mm. after 4 weeks. Not complete after 12 weeks.

Pigment—"Naphthalene Yellow" after 4 weeks.

Potato plug.

Growth—good, nodular, then echinate, acervate; on lower part—(1) "Smoke Gray"; (2) "Dark Olive Buff"; (3) "Buffy Brown"; on upper part "Olive Brown."

A.m.—(1) scant; (2) fair, mainly on upper part. "Pale Mouse Gray" with whitish spots.

Plug—(1) "Hair Brown"; (2) "Wood Brown."

Carrot plug. (Fig. 6 D.)

Growth—good, nodulose becoming echinate. "Pale Smoke Gray"; margins very puckered. Plug very shrivelled after 4 weeks.

A.m.—abundant after 3 weeks. White.

Colour of plug—"Russet."

Tyrosinase reaction—positive, fairly strong. Anaerobic growth—none.

Starch plate—positive. Width of hydrolytic zone = 2 mm. Width of incomplete zone = 5 mm.

Nitrate reduction—positive 2. Width of colour zone = 3 mm.

Growth at 37·5° C.—fairly good.

Liquid Media.

Saccharose synthetic solution.

Growth—fairly good, at base only. (1) Numerous compact colonies with smooth margins; (2) soft spongy masses.

Glycerine synthetic solution. (Fig. 7 B.)

Growth—poor, at base only. (1) Colonies with smooth margins; (2) less regular, coalescing.

Glucose broth.

Growth—fairly good, at base only, spongy mass.

Pigment—deep golden brown.

Starch synthetic solution.

Growth—on surface—none. In medium—heavy growth of large, soft, spherical colonies forming a thick layer at base resembling tapioca.

Hydrolysis—supernatant liquid clear.

Brom-cresol milk.

Growth—surface, heavy, wrinkled—"Dutch Blue" with rim of "Cinnamon Brown."

A.m.—none.

Coagulation—positive after 3 weeks.

Digestion of clot—20 mm. after 3 weeks; final 30 mm. (cloudy).

Colour of digestion zone—(1) "Green Blue Slate"; (2) "Blue Violet Black."

Strains 18 and 19 = *Actinomyces clavifer*.

Morphology—on egg albumen agar culture—3 weeks old.

Conidiophore. Much branched. Sporulation starts at the tips of the branches before segmentation has become general; long sporogenous branches eventually form. Some filaments terminate in club-shaped structures.

Conidia. Large, cylindrical. $1{\cdot}5 \times 1{\cdot}0\mu$. (Fig. 2.)

Cultural characteristics.

Solid Media.

Saccharose synthetic agar. (Fig. 5 A.)

Colonies—small, umbonate, some "Pale Smoke Gray"; others "Brick Red."

Streak—acervulate. "Brick Red," colour more obvious from reverse side.

A.m.—(1) scant; (2) abundant. (1) White; (2) white sprinkled with "Light Cinnamon Drab."

Guttation—minute, colourless drops after 4 weeks.

Pigment—(1) "Ivory Yellow"; (2) golden brown; (3) deep golden brown.

Glycerine synthetic agar. (Fig. 5 B.)

Colonies—small, slightly raised, good growth in substratum.

Streak—good, granular. "Apricot Orange" from reverse side.

A.m.—(1) fair; (2) abundant. (1) White; (2) white mixed with "Light Cinnamon Drab."

Pigment—(1) "Ivory Yellow"; (2) "Brick Red"; (3) blackish red.

Dextrose synthetic agar. (Fig. 5 C.)

Streak—(1) poor, thin, flat on surface, the major portion of growth in substratum; "Pale Smoke Gray"; (2) irregular humps appear along the streak which are (*a*) "Snuff Brown"; (*b*) "Cinnamon Brown"; (3) good, rugose, "Warm Blackish Brown" deepening.

A.m.—(1) appears on all raised parts; white to "Light Drab"; (2) good, but uneven; (1) "Light Drab"; (2) white.

Pigment—(1) "Cinnamon Buff"; (2) "Orange Cinnamon"; (3) "Mikado Brown" deepening.

Calcium malate glycerine agar. (Fig. 6 B.)

Streak—good, flat, biting into medium. Margins crenate. (1) "Ochraceous Orange," centres of colonies turning "Cinnamon Brown"; (2) deep blackish brown at base (where not covered by A.m.).

A.m.—(1) scant; (2) fairly good. (1) "Light Ochraceous Salmon"; (2) "Drab Gray" changing to "Light Cinnamon Drab."

Guttation—slight.

Pigment—(1) "Pale Ochraceous Buff"; (2) "Hay's Russet"; immediately below growth.

Dextrose agar (Krainsky's).

Streak—(1) fair, colonies flat, biting into medium; (2) good, rough pimply appearance. (1) "Brick Red"; (2) "Warm Blackish Brown"; (3) black. Margins laciniate.

A.m.—(1) fair; (2) abundant, filling up hollows in growth. (1) White to "Light Cinnamon Drab"; (2) whitish.

Guttation—slight, colourless, ceasing after 3 weeks.

Pigment—(1) golden brown; (2) deep golden brown.

Nutrient potato agar. (Fig. 6 C.)

Growth—good, nodulose, wrinkled. (1) "Pale Smoke Gray"; (2) "Deep Grayish Olive."

A.m.—trace.

Pigment—deep golden brown.

Egg albumen agar. (Fig. 7 A.)

Streak—good, flat, biting into medium—colonies have raised centres. (1) "Orange Rufous," nearly black where the growth is strongest; (2) "Bay"; (3) reddish black.

A.m.—scant, on the centres of colonies. (1) White; (2) "Pale Olive Gray" with some older parts "Olivaceous Black"; (3) white.

Pigment—(1) "Orange Rufous"; (2) "Mahogany Red."

Gelatine.

Growth—(1) poor; (2) good. (1) "Pale Smoke Gray"; (2) "Colonial Buff."

A.m.—covering surface growth. White.

Liquefaction—napiform. 20 mm. digested in 4 weeks. Not complete after 12 weeks.

Pigment—(1) "Yellow Ochre"; (2) reddish yellow.

Potato plug.

Streak—good, echinate, wrinkled, acervate. (1) "Pale Smoke Gray" changing to "Orange Cinnamon"; (2) "Warm Blackish Brown."

A.m.—(1) scant; "Pale Mouse Gray"; (2) good. (1) "Neutral Gray" on lower part; "Olive Buff" on upper part; (2) "Pallid Neutral Gray" on lower part; "Olive Buff" on upper part.

Guttation—very slight or absent.

Colour of plug—(1) "Drab Gray"; (2) "Natal Brown."

Carrot plug. (Fig. 6 D.)

Colonies—raised, wrinkled. (1) "Pale Smoke Gray"; (2) "Tawny" to "Orange Cinnamon."

Streak—fairly good. (1) "Chocolate"; (2) "Warm Blackish Brown."

A.m.—fairly good, late. Whitish.

Colour of plug—(1) "Natal Brown"; (2) "Bone Brown." Plug very shrivelled.

Tyrosinase reaction—positive. Anaerobic growth—none.

Starch plate—positive. Width of hydrolytic zone = 3 mm. Width of incomplete zone = 5 mm.

Nitrate reduction—negative. Growth at 37·5° C.—fair.

Liquid Media.

Saccharose synthetic solution.

Growth—good. (1) Numerous colonies each with dark spot in centre, on surface, clinging to sides of tube, and collected at base. No colonies suspended in medium. (2) All colonies star-like. Surface growth abundant. Surface colonies and centres of side colonies "Vinaceous Cinnamon."

A.m.—good. (1) White; (2) "Drab Gray."

Pigment—(1) light golden brown; (2) deep golden brown.

Glycerine synthetic solution. (Fig. 7 B.)

Growth—good. (1) Small compact colonies with laciniate margins mainly at base, with some clinging to sides of tube; (2) same with some surface colonies; (3) colonies larger and star-like and evenly distributed. Dark spot in centre, "Colonial Buff." Surface colonies (1) "Salmon Colour"; (2) "Verona Brown"; (3) "Warm Blackish Brown."

A.m.—very scant. (1) "Light Drab"; (2) "Warm Sepia."

Pigment—(1) "Cartridge Buff"; (2) "Cream Buff"; (3) old gold, deepening.

Glucose broth.

Growth—fairly good; (1) sponge-like mass at base and a few colonies on sides of tube. A few colonies may appear on surface, but all growth ultimately sinks to the bottom.

A.m.—none.

Pigment—very deep golden brown.

Starch synthetic solution.

Growth—on surface—(1) floating colonies half submerged, small, round; (2) some colonies showing irregularly rolled appearance resembling the droppings of birds. "Ochraceous Buff" or "Mummy Brown." In medium—numerous, very large, soft, spherical colonies (as in Strain 17) later, tending to break up.

A.m.—fair—white shading to "Vinaceous Buff."

Guttation—slight.

Hydrolysis—supernatant liquid clear.

Pigment—(1) "Cream Colour"; (2) old gold.

Strain 20 = *Actinomyces praecox*.

Morphology.

Conidiophore. Branching irregular, the longer branches forming widely open spirals. Sporulation starts at the apices of the terminal branches.

Conidia. Spherical or oval. $0{\cdot}85 \times 0{\cdot}83\mu$. (Fig. 2.)

Cultural characteristics.

Solid Media.

Saccharose synthetic agar. (Fig. 5 A.)

Streak—(1) fair; (2) good, thin, flat, powdery. Margins finely laciniate, fringe-like.

A.m.—(1) in minute spots; (2) in thin layer. (1) Whitish; (2) "Pale Olive Buff."

Glycerine synthetic agar. (Fig. 5 B.)

Streak—good, flat, mealy; margins laciniate.

A.m.—abundant, appears with growth. (1) White; (2) "Pale Olive Buff" on lower part only.

Pigment—"Ivory Yellow."

Dextrose synthetic agar. (Fig. 5 C.)

Streak—good, echinate. Margins ribbon-like, flat. (1) Crenate; (2) laciniate.

A.m.—appears with growth, abundant. (1) White in centre, "Olive Buff" along edges; (2) whitish in centre, "Grayish Olive" along edges.

Guttation—minute colourless drops appearing in first week and ceasing after third week.

Pigment—"Cream colour."

Calcium malate glycerine agar. (Fig. 6 B.)

Streak—good, thin, flat, spreading, biting into medium. "Pale Smoke Gray." Margins crenate.

A.m.—abundant. Mealy. (1) "Olive Buff"; (2) "Marguerite Yellow."

Guttation—colourless drops.

Pigment—"Marguerite Yellow" in 4th week.

Dextrose agar (Krainsky's).

Streak—good, flat, somewhat mealy. Margins laciniate.

A.m.—abundant, appears with growth. (1) "Light Grayish Olive"; (2) "Deep Olive Buff."

Nutrient potato agar. (Fig. 6 C.)

Streak—good, echinate; margins (1) crenate; (2) finely fringed; streak later becomes smoother and marginal fringe tends to disappear.

A.m.—appears with growth, abundant. White.

Egg albumen agar. (Fig. 7 A.)

Streak—good, thin, flat, spreading, biting into medium. "Pale Smoke Gray." Margins laciniate.

A.m.—(1) fair, scattered, powdery appearance; (2) good. "Olive Buff."

Gelatine.

Growth—good.

A.m.—abundant. White.

Liquefaction—stratiform 25 mm. digested in 4 weeks. Complete in 9 weeks.

Potato plug.

Growth—good, finely echinate.

A.m.—abundant, appears with growth. (1) White; (2) "Pale Olive Buff."

Guttation—(1) in 1st week minute colourless drops; (2) abundant large drops—(1) "Ivory Yellow"; (2) "Deep Olive Buff." Ceases after 4 weeks.

Colour of plug—(1) "Deep Olive Buff"; (2) "Drab."

Carrot plug. (Fig. 6 D.)

Growth—good, fine, smooth.

A.m.—good, appears with growth, fine, mealy. (1) "Olive Buff"; (2) "Pale Olive Buff."

Colour of plug—unchanged.

Tyrosinase reaction—negative. Anaerobic growth—none.

Starch plate—positive. Width of hydrolytic zone = 10 mm. Width of incomplete zone = 12 mm.

Nitrate reduction—negative. Growth at 37·5° C.—good.

Liquid Media.

Saccharose synthetic solution.

Growth—fair; (1) soft flaky masses throughout medium; (2) sinking to base of tube.

A.m.—none.

Glycerine synthetic solution. (Fig. 7 B.)

Growth—good. (1) Surface growth with flakes throughout medium and at base of tube; (2) heavy growth at surface in a film-like mass; a little at base.

A.m.—abundant, powdery, white.

Guttation—good; colourless drops.

Pigment—"Pale Chalcedony Yellow" after 4 weeks.

Glucose broth.

Growth—good; (1) flocculent bottom growth, a heavy surface growth, and a number of sponge-like colonies suspended in medium; (2) all growth comes to the surface, very wrinkled.

A.m.—abundant. White.

Starch synthetic solution.

Growth—on surface, good; (1) flat, thin; (2) very heavy, forming thick uneven film from which filmy flakes hang into the medium. In medium—(1) heavy, soft flaky colonies; (2) bulky flaky mass.

A.m.—abundant; (1) on the centre and rims of colonies; (2) covers growth. White to "Pale Chalcedony Yellow."

Guttation—copious. Minute colourless drops. As these disappear concentric circles are formed around pin-point depressions.

Hydrolysis—supernatant liquid clear.

Pigment—(1) "Cream colour"; (2) "Yellowish Olive."

Brom-cresol milk.

Growth—surface—good. Finally "Slate Blue." In medium—flocculent masses.

A.m.—ring—white.

Coagulation—positive.

Digestion of clot—20 mm. after 2 weeks. Final, complete.

Colour of digestion zone—(1) "Dull Dusky Purple"; (2) "Dark Hyssop Violet"; (3) "Mulberry Purple."

Smell—the peculiar and penetrating smell of the *Actinomyces* group is particularly strong in this species.

Strain 21 = *Actinomyces flavus* (Millard and Burr).

Morphology.

Conidiophore. Simple, slightly branched. Sporulation regular.

Conidia. Barrel-shaped; germination whilst still attached frequent. 1·31 to 0·87 $\mu \times 0{\cdot}86$ to 0·6 μ. (Fig. 2.)

Cultural characteristics.

Solid Media.

Saccharose synthetic agar. (Fig. 8 A.)

Streak—fairly good, thin, flat, larger colonies slightly raised. Margins laciniate at first. Substratum growth—fringe-like.

A.m.—(1) scant, on raised colonies; (2) fair. (1) "Mouse Gray"; (2) "Mouse Gray" speckled with white.

Pigment—after 3rd week in upper part of slope spreading downwards. "Chalcedony Yellow."

Dextrose synthetic agar. (Fig. 8 B.)

Streak—very good. Smooth to echinate. Margins laciniate. Substratum growth—fringe-like.

A.m.—abundant. (1) "Deep Mouse Gray" with specks of white; (2) "Dark Mouse Gray"; (3) "Dark Quaker Drab."

Guttation—slight, then good.

Pigment—(1) "Chartreuse Yellow"; (2) "Straw Yellow."

Calcium malate glycerine agar. (Fig. 8 C.)

Streak—flat, thin, slightly raised edges.

A.m.—abundant, powdery. (1) "Light Chalcedony Yellow"; (2) "Primrose Yellow"; (3) "Barium Yellow."

Pigment—corresponding in each stage to colour of A.m.

Nutrient potato agar.

Growth—colonies large, raised, convex, slowly forming streak. Margins very flat, thin, finely crenate.

A.m.—abundant, velvety—very early. (1) White; (2) "Neutral Gray," each colony rimmed with white; (3) "Deep Quaker Drab."

Egg albumen agar. (Fig. 8 D.)

Streak—good, thin, flat. Margins slightly raised, laciniate. Substratum growth feathery.

A.m.—fairly good. (1) "Light Olive Gray" with "Mouse Gray" edging; (2) all "Light Olive Gray."

Pigment—"Pale Dull Green Yellow."

Gelatine.

Growth—on surface—good, plicate.

A.m.—covering growth. White.

Liquefaction—napiform, then stratiform. 13 mm. in 4 weeks.

Potato plug.

Growth—good. Echinate to wrinkled.

A.m.—abundant. (1) "Deep Quaker Drab"; (2) "Dark Quaker Drab" with whitish edging.

Guttation—good.

Colour of plug—unchanged.

Tyrosinase reaction—negative. Anaerobic growth—none.

Starch plate—positive. Width of hydrolytic zone = 6–7 mm.

Nitrate reduction—positive after 3 days; negative after 1 week.

Growth at 37·5° C.—good.

Liquid Media.

Saccharose synthetic solution. (Fig. 8 F.)

Growth—(1) scant; (2) fairly good; on surface (1) scant; (2) fair in medium, flaky colonies suspended, clinging to the sides of the tube, and accumulating at the bottom.

A.m.—scant. (1) White; (2) "Mouse Gray."

Glycerine synthetic solution. (Fig. 8 E.)

Growth—good. (1) Speck-like colonies cover surface and are suspended through the medium; (2) most of growth collects at the surface and the remainder in a flaky mass at the bottom, leaving medium clear. Colour of surface growth (1) "Deep Colonial Buff"; (2) "Olive Ochre."

A.m.—fair. (1) White; (2) "Primrose Yellow."

Guttation—slight.

Pigment—(1) "Light Chalcedony Yellow"; (2) light yellow.

Glucose broth. (Fig. 8 H.)

Growth—good. At surface, large colonies form, coalesce, and sink very slowly; medium remains clear.

A.m.—fair. White.

Pigment—pinkish brown.

Dextrose synthetic solution. (Fig. 8 G.)

Growth—good on surface. (1) Discrete colonies; (2) thick layer, echinate. Upper edge—yellow; lower edge—"Russet." In medium—suspended flakes disappearing leaving medium clear; at bottom—(1) flocculent mass; (2) slight, flaky.

A.m.—good. (1) "Light Olive Gray"; (2) "Deep Olive Gray."

Guttation—fair.

Pigment—light golden brown.

Brom-cresol milk.

Growth—fairly good.

Coagulation—positive.

Digestion of clot—35 mm. in 3 weeks, further change very slow.

Colour of digestion zone—(1) "Dull Dark Purple"; (2) "Livid Purple"; (3) "Perilla Purple."

Strain 22 = *Actinomyces craterifer*.

Morphology.

Conidiophore. Much branched, terminal branches often dichotomously forked. Sporulation regular. Meta-chromatic granules numerous in main axis.

Conidia. Rectangular to square. $1{\cdot}3\mu$ to $0{\cdot}9 \times 1{\cdot}0$ to $0{\cdot}8\mu$. (Fig. 2.)

Cultural characteristics.

Solid Media.

Saccharose synthetic agar. (Fig. 8 A.)

Streak—good, finely echinate. Margins laciniate. Substratum growth—softly fringe-like.

A.m.—good. (1) "Light Mouse Gray"; (2) "Deep Mouse Gray."

Guttation—copious, minute drops, very early. As the droplets disappear minute blackish craters are left behind.

Dextrose synthetic agar. (Fig. 8 B.)

Streak—very good, finely echinate. Margins (1) flat, crenate; (2) fringed. Substratum growth—fringe-like.

A.m.—abundant. "Deep Mouse Gray" flecked with "Light Mouse Gray."

Guttation—not copious, but leaving surface covered with crater-like pin-holes.

Pigment—trace.

Calcium malate glycerine agar. (Fig. 8 C.)

Colonies—flat with pimple-like centres.

Streak—good, flat, thin. Margins soft, downy. Substratum growth—(1) bowl-like; (2) softly feathery.

A.m.—(1) scant, appearing first on centres of colonies; (2) good. (1) "Olive Gray" speckled with white; (2) "Neutral Gray" speckled with white. In many colonies points of A.m. also arise on the margins, giving a fairy ring appearance.

Nutrient potato agar.

Streak—good. Colonies flat with raised centres, retaining their shape; glistening. "Pale Smoke Gray." Margins flat.

A.m.—scant, first on centres of colonies; scattered. White.

Egg albumen agar. (Fig. 8 D.)

Streak—fairly good. Colonies flat, with raised centres retaining shape. Margins, softly downy. Substratum growth—good—(1) bowl-like; (2) softly fringe-like.

A.m.—(1) scant; (2) good, on centres of colonies. (1) White; (2) "Pale Smoke Gray"; (3) "Mouse Gray."

Gelatine.

Growth—beaded, with few large colonies. Wrinkled on surface.
A.m.—good. White.
Liquefaction—napiform, then stratiform. 20 mm. in 4 weeks.

Potato plug.

Growth—good, echinate.
A.m.—abundant. (1) White; (2) white, speckled with "Light Mouse Gray" turning to "Mouse Gray" and lastly to "Quaker Gray."
Guttation—copious.
Colour of plug—unchanged.

Tyrosinase reaction—negative. Anaerobic growth—none.

Starch plate—positive. Width of hydrolytic zone = 5–6 mm.

Nitrate reduction—positive after 3 days. Negative after 1 week.

Growth at 37·5° C. = slight.

Liquid Media.

Saccharose synthetic solution. (Fig. 8 F.)

Growth—fairly good, on surface, rim of colonies; in medium, numerous colonies attached to the sides of the tube by their fringed margins, from which a filmy net-like growth floats into the liquid; at base a filmy mass of colonies.
A.m.—fairly good. (1) White; (2) "Pale Mouse Gray"; (3) "Mouse Gray."

Glycerine synthetic solution. (Fig. 8 E.)

Growth—fairly good. On surface—rim of colonies. In medium—numerous star-like colonies clinging to side of tube. At base—discrete colonies becoming flaky.
A.m.—scant. (1) White; (2) "Pale Mouse Gray."

Glucose broth. (Fig. 8 H.)

Growth—fair. On surface—slight at first. Medium clear. Bottom growth softly compact; later, rising to surface.
A.m.—fair. White to "Pale Smoke Gray."
Guttation—slight or absent.

Dextrose synthetic solution. (Fig. 8 G.)

Growth—good. On surface—piled up, fringed. In medium—colonies clinging to side of tube, very fringed, each showing a dark core. Bottom—mass of softly compact colonies.
A.m.—good. (1) White; (2) "Pale Olive Gray"; (3) "Light Grayish Olive"; (4) "Mouse Gray" still showing some white.
Guttation—slight.

Brom-cresol milk.

Growth—good, falling to bottom.
Coagulation—none.
Hydrolysis—rapid. Complete in 3 weeks.
Colour of digestion zone—(1) "Varley's Gray"; (2) "Dull Bluish Violet (1)"; (3) "Dark Hyssop Violet."

Strain 23 = *Actinomyces fimbriatus.*

Morphology (from culture on saccharose synthetic agar).

Conidiophore. Much branched, the lateral branches frequently forming spirals of 3 or more turns. Sporulation regular, starting in the lower branches. Meta-chromatic granules numerous in main axis.

Conidia. Cylindrical to oval, very variable in length. 1·16 to 0·85μ × 0·88μ. (Fig. 2.)

Cultural characteristics.

Solid Media.

Saccharose synthetic agar. (Fig. 8 A.)

Streak—good, convolute. "Pale Smoke Gray." Margins (1) flat; (2) laciniate; (3) tomentose.

A.m.—abundant. (1) White; (2) "Mouse Gray" with a few specks of white.

Guttation—slight after 3 weeks.

Pigment—"Cream Colour" after 4 weeks.

Dextrose synthetic agar. (Fig. 8 B.)

Streak—very good, echinate. Margins (1) flat, soft; (2) fringed.

A.m.—good. (1) White; (2) "Pallid Mouse Gray"; (3) "Mouse Gray" flecked with "Pallid Mouse Gray."

Guttation—fair, very minute drops.

Calcium malate glycerine agar. (Fig. 8 C.)

Streak—(1) fairly good, discrete colonies, flat with raised centres; (2) good, thin. "Avellaneous." Margins (1) laciniate; (2) heavily fringed. Substratum growth—(1) bowl-like; (2) feathery.

A.m.—very scant, appearing late. (1) White; (2) "Pallid Purplish Gray."

Nutrient potato agar.

Colonies—flat, raised centres, not coalescing. (1) "Pale Smoke Gray"; (2) blackish. Margins laciniate.

A.m.—a few specks. White.

Pigment—(1) deep golden brown; (2) very deep golden brown.

Egg albumen agar. (Fig. 8 D.)

Colonies—flat, with pin-point raised centres.

Streak—good. Margins laciniate, then heavily fringed. Substratum growth—(1) bowl-like; (2) fringe-like.

A.m.—(1) scant; (2) good. (1) White; (2) turning to "Pale Smoke Gray."

Gelatine.

Growth—fairly good, beaded, boring into medium in crateriform manner forming hole 7 mm. deep, 10 mm. wide.

A.m.—white.

Liquefaction—none after 4 weeks.

Pigment—"Corinthian Red."

Potato plug.

Growth—good. "Dark Mouse Gray."

A.m.—scant, on dried up portion only. (1) White; (2) "Mouse Gray."

Colour of plug—"Sooty Black" around growth.

Tyrosinase reaction—strongly positive. Anaerobic growth—none.
Starch plate—positive. Width of hydrolytic zone = 2–3 mm.
Nitrate reduction—positive 3. Growth at 37·5° C.—good.

Liquid Media.

Saccharose synthetic solution. (Fig. 8 F.)

Growth—on surface—very slight or none. In medium—numerous large colonies clinging to side of tube, margins fringed. At base—softly compact mass. After 6 weeks all growth at base.

A.m.—none.

Glycerine synthetic solution. (Fig. 8 E.)

Growth—on surface—numerous colonies (1) covering surface, (2) forming rim, which sinks in masses to the bottom. In medium—numerous colonies clinging to side of tube. At base—mass of compact colonies.

A.m.—scant, white, disappearing as colonies sink.

Glucose broth. (Fig. 8 H.)

Growth—fairly good—mainly at base, flocculent mass; one or two colonies form on surface and a few float in the medium.

A.m.—none.

Pigment—(1) golden brown; (2) dark golden brown.

Dextrose synthetic solution. (Fig. 8 G.)

Growth—good on surface. A wrinkled skin-like mass which tends to sink. In medium—colonies cling to sides of tube but later, sink to bottom. At base—sponge-like mass.

A.m.—none.

Brom-cresol milk.

Growth—good. Surface rim—"Hay's Maroon."

Coagulation—none.

Hydrolysis—none.

Colour of digestion zone—(1) "Light Payne's Gray"; (2) "Dark Green Blue Gray"; (3) "*5. Slate Gray"; (4) "Dark Purplish Gray."

KEY FOR IDENTIFICATION OF THE SPECIES DESCRIBED.

Growth in Glycerine Synthetic Solution.

1. Star-like colonies which persist in suspension or clinging to the sides of tube.

   Deep pigment produced in nearly all media.
   Tyrosinase reaction +; forms solid curd in brom-cresol milk. *A. clavifer.*

   Deep pigment produced in protein media only.
   Tyrosinase reaction +. *A. fimbriatus.*

   Pale pigment produced in nearly all media.
   Tyrosinase reaction −. *A. carnosus.*

   No pigment (or only trace) produced in media.
   Growth on saccharose and dextrose media covered with minute craters.
   A. craterifer.

2. Heavy surface growth with abundant aerial mycelium.

Aerial mycelium on nutrient potato agar, not abundant, "Pale Smoke Gray," forming fern-like outgrowths from margin of growth on saccharose synthetic agar. *A. gracilis.*

Aerial mycelium on nutrient potato agar abundant, white; liquefaction of gelatine—stratiform with no pigment in medium; matures quickly on all media producing abundant aerial mycelium. *A. praecox.*

liquefaction of gelatine—napiform producing pigment in medium; pigment in calcium malate glycerine agar "Chamois." *A. Setonii.*

pigment in calcium malate glycerine agar "Carnelian Red"; quickly produces abundant aerial mycelium on nearly all media. *A. praefecundus.*

3. Fair surface growth with some aerial mycelium.

Produces pigment (often green) on all solid media. *A. viridis.*

Produces pigment (yellow) on all synthetic media. *A. flavus* (M. and B.).

Seldom produces pigment and then poor; forms a decided clot in brom-cresol milk. *A. Wedmorensis.*

4. Scant or no surface growth, but some bottom growth.

Produces colour changes in brom-cresol milk.

Tyrosinase reaction + ; produces no aerial mycelium on nutrient potato agar. *A. scabies* (Thaxter) Güssow (M. and B.).

Tyrosinase reaction − ; produces aerial mycelium on most solid media.

Abundant aerial mycelium on saccharose synthetic agar.

Good growth with abundant aerial mycelium on egg albumen agar. *A. tenuis.*

Poor growth with scant aerial mycelium on egg albumen agar. *A. marginatus.*

Scant aerial mycelium on saccharose synthetic agar.

Produces no true aerial mycelium on any media. Colonies often show dark centres. *A. salmonicolor.*

Produces no colour changes in brom-cresol milk.

Facultative anaerobe; produces no true aerial mycelium or only trace on any media. The colonies frequently show dark centres. *A. maculatus.*

Obligate aerobe; the aerial mycelium arises centripetally on the colonies. *A. coroniformis.*

5. No growth. Starch not hydrolysed.

Good growth in brom-cresol milk with characteristic colour changes. *A. spiralis.*

Poor growth in brom-cresol milk with no coagulation, no hydrolysis and no colour change. *A. Sampsonii.*

Sub-cultures of the above species have been sent to the Lister Istitute, Chelsea, S.W. 1.

SUMMARY.

The difficulty of reconciling various cultures of the scab organism with any one defined type of *A. scabies* (Thaxter) Güssow is discussed.

Inclusion of all such cultures into one *A. scabies* group has no justification.

A technique has been evolved whereby the cultural characters of *Actinomyces* may be maintained in sub-culture.

Twenty-four *Actinomyces* strains were isolated from potato scabs and other sources, and the cultural and morphological characters of these have been worked out and described.

Three pairs of duplicate strains appeared, but with these exceptions, the differences between the strains were of specific rank.

One of the species appears to be identical with Thaxter's original scab organism and is thus referred to *A. scabies* (Thaxter) Güssow; the remainder are new and have accordingly been named.

Eleven of the species were found to produce scab on potatoes.

The various types of common scab have been defined, and it has been shown that type of scab is dependent on the infecting *Actinomyces* species. The variety of potato may modify but cannot materially change this type.

A. scabies (Thaxter) Güssow (emend. M. and B.) produced a deeply furrowed scab apparently identical with the "Deep Scab" described by Thaxter. This species was also found to attack the roots and stolons of potato plants.

The occurrence of scab in virgin soils and the predominance of certain scab types in certain soils and in different years are discussed.

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EXPLANATION OF PLATES XV—XX

PLATE XV.

Fig. 3. (*a*) "Pitted" scab. A naturally infected potato from which Strain 17 was isolated.
(*b*) "Pitted" scab produced by inoculation with Strain 17—Variety "Ally."
(*c*) "Pitted" scab produced by inoculation with Strain 17—Variety "Great Scot."
(*d*) Stunted roots of potato plant inoculated with Strain 17 showing dark nodular growths of *Actinomyces.*
(*e*) "Pimple" scab produced by inoculation with Strain 15.

PLATE XVI.

Fig. 4. (*a*) "Knob" scab. A naturally infected potato from which Strain 20 was isolated.
(*b*) "Tumulus" scab. A naturally infected potato in which the early stage (a conical depression) and the later stage (a mound-like swelling) are seen. Strain 21 was isolated from such a tuber.
(*c*) "Tumulus" scab produced by inoculation with Strain 21 and showing the typical conical depression of the early stage. Upward swelling has started in most of the scabs.
(*d*) "Pitted" scab produced by inoculation with Strain 9.
(*e*) "Pitted" or pock-like scab produced by inoculation with Strain 23.

PLATE XVII.

Fig. 5. *A* 1–20. Cultures on saccharose synthetic agar.
B 1–12 and 17–20. Cultures on glycerine synthetic agar.
C 17–20. Cultures on dextrose synthetic agar.

In Plates XVII–XX the number below each tube is that of the Strain.

PLATE XVIII.

Fig. 6. *A* 1–12. Cultures on dextrose synthetic agar.
B 13–20. Cultures on calcium malate glycerine agar.
C 1–12 and 17–20. Cultures on nutrient potato agar
D 17–20. Cultures on carrot plug.

PLATE XIX.

Fig. 7. *A* 1–20. Cultures on egg albumen agar.
B 1–20. Cultures on glycerine synthetic solution.

PLATE XX.

Fig. 8. *A* 21–23. Cultures on saccharose synthetic agar.
B 21–23. Cultures on dextrose synthetic agar.
C 21–23. Cultures on calcium malate glycerine agar.
D 21–23. Cultures on egg albumen agar.
E 21–23. Cultures on glycerine synthetic solution.
F 21–23. Cultures on saccharose synthetic solution.
G 21–23. Cultures on dextrose synthetic solution.
H 21–23. Cultures on glucose broth.

The age of the cultures in Plates XVII–XIX is 4–5 weeks and of those in Plate XX 6 weeks.

Note. The blocks for the above plates have been kindly lent by the University of Leeds and the Yorkshire Council for Agricultural Education.

(*Received November 12th*, 1925.)

Fig. 3.

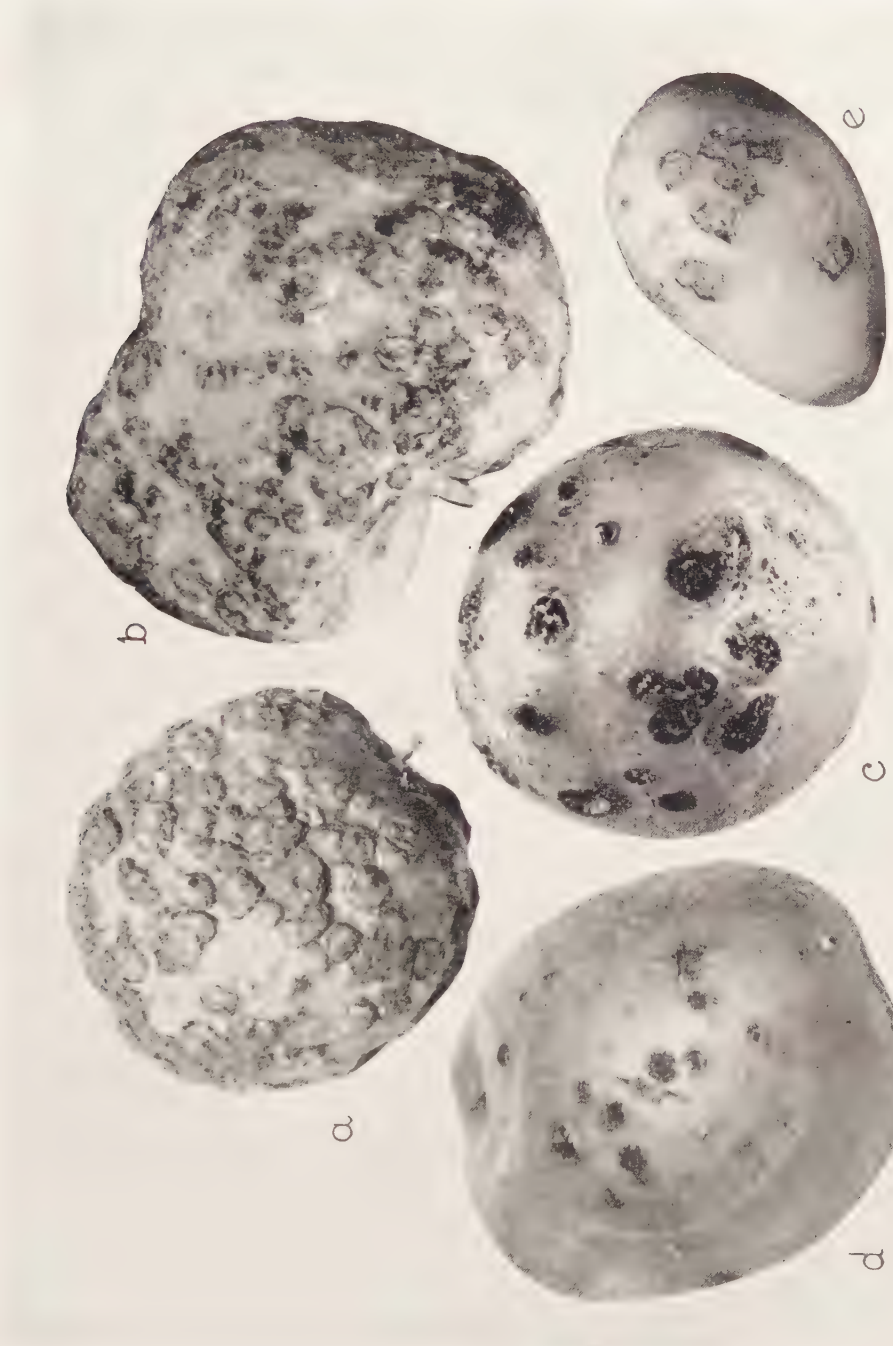
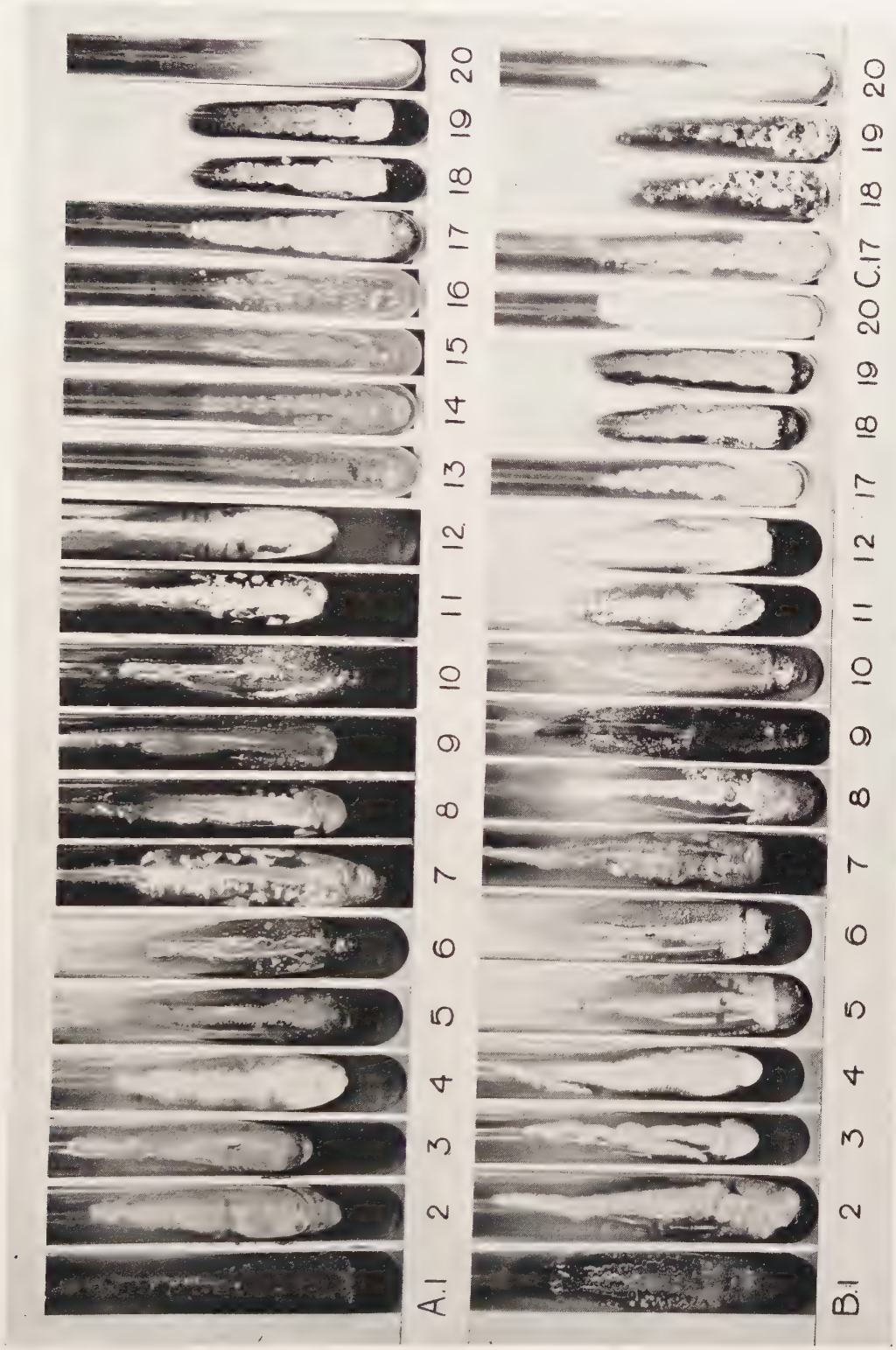


Fig. 4.

MILLARD & BURR.—A Study of Twenty-Four Strains of *Actinomyces*. (pp. 580—644.)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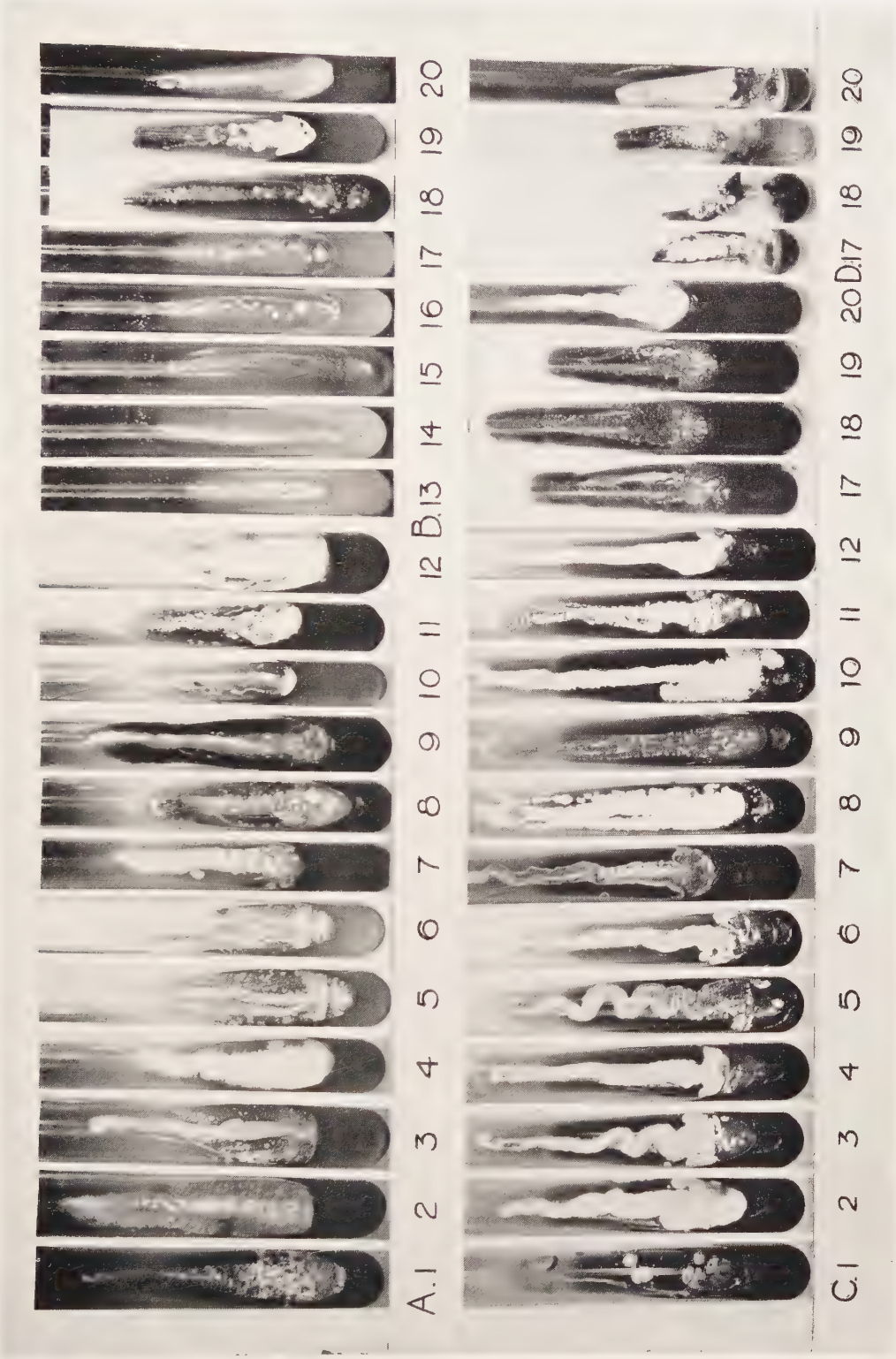
B.1 2 3 4 5 6 7 8 9 10 11 12 17 18 19 20 C.17 18 19 20

Fig. 5.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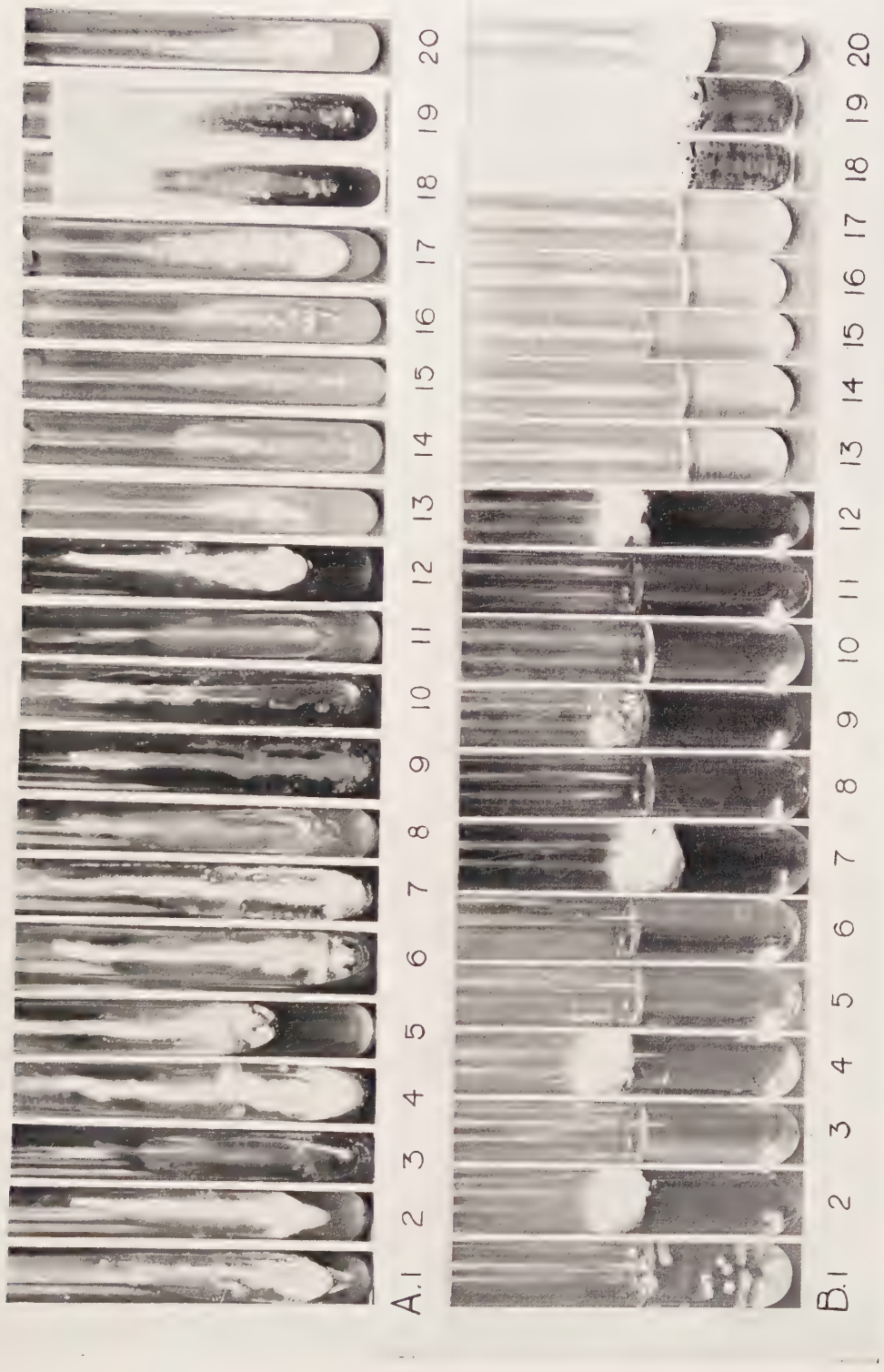
C.1 2 3 4 5 6 7 8 9 10 11 12 17 18 19 20 D.17 18 19 20

Fig. 6.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B.1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Fig. 7.

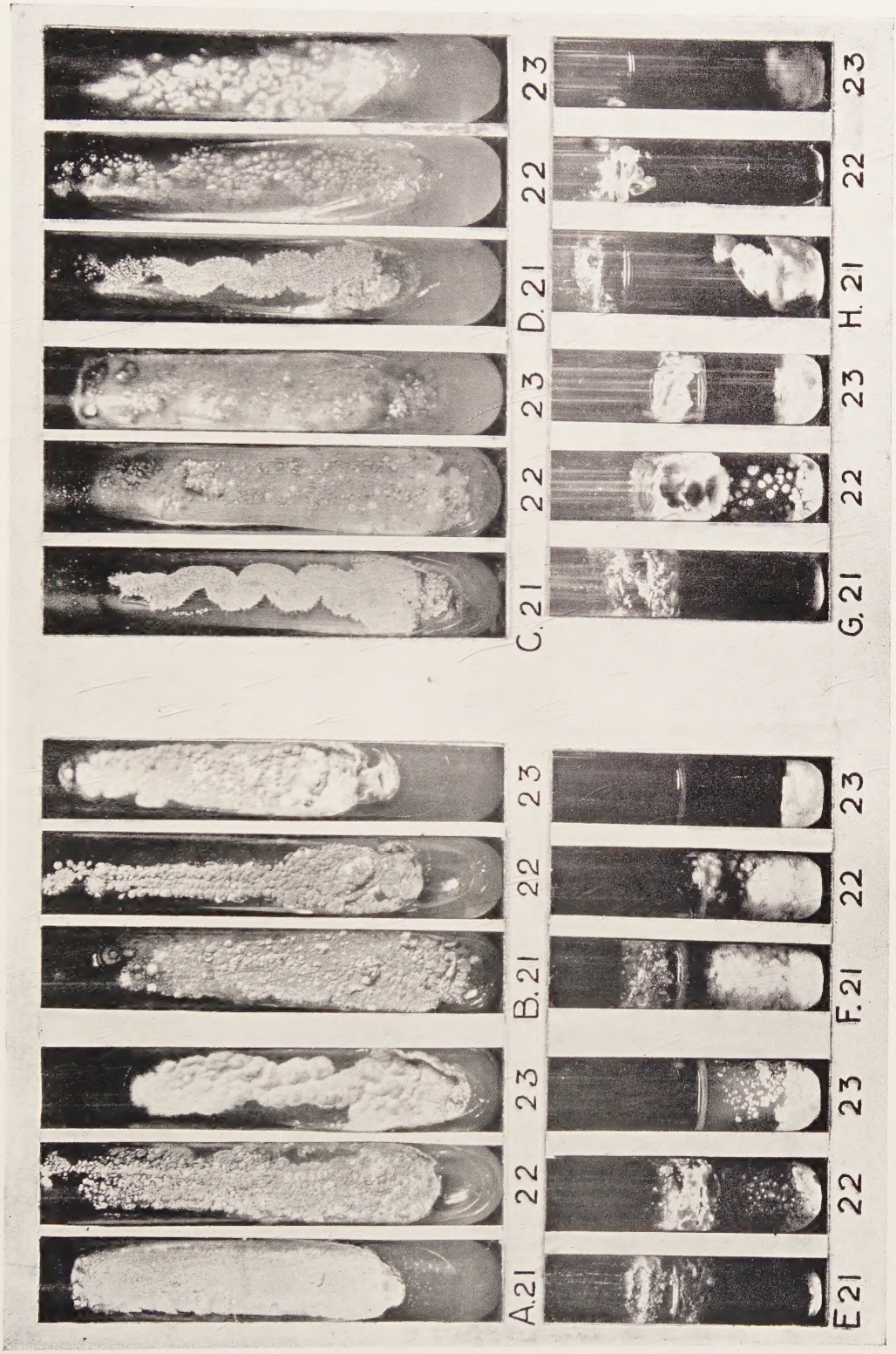

Fig. 8.

MILLARD & BURR.—A Study of Twenty-Four Strains of *Actinomyces*. (pp. 580—644.)

REVIEW

Die Ökologie der Blattminierenden Insektenlarven. Von Dr Martin Hering. Zoologische Bausteine: Ausschnitte aus dem Gesamtgebiet der Zoologie, Band 1, Heft 2. Pp. iv + 253 + 2 Tafeln. (Berlin: Gebrüder Borntraeger, 1926.) 18 gold marks.

The above treatise by Dr Hering is the product of a number of years' investigation of a specialised branch of entomology. In this work he has collated and gathered together the large amount of information relative to leaf-mining insect larvae that lies scattered through the pages of dozens of periodicals. At the same time, by his own studies and generalizations, he has infused greater exactness into a study that has hitherto remained ill-defined and in need of more scientific treatment. We believe that he can claim to be the first to review the leaf-mining habit as an ecological entity and to correlate the diverse types of mine-formation with leaf-structure. In his effort to formulate his subject, Dr Hering has made use of a special terminology for the purpose of differentiating various characteristic features of mines. It may be added, however, that the words he has coined are convenient and on the whole euphonious and, for this reason, we think that they may find favour with subsequent workers in the same field. The first chapter is concerned with the "Definition and Morphology of the Mine." With the aid of illustrations of plant tissue in cross-section he shows that mines may be restricted either to the spongy or palisade parenchyma or extend through both those layers of tissue: on the other hand there is also figured an epidermal mine, or one which leaves the assimilating tissue unaffected. By adopting the root *nom* (from ὑπόνομος, a mine in the sense of a tunnel in the earth) and the addition of various prefixes, he distinguishes a stem-mine as a *caulonom*: a mine in a fruit as a *carponom*: a flower-mine as an *anthonom*, and a leaf-mine as a *phyllonom*. Wherever the mines may be situated they exhibit definite morphological characters. Thus, a serpentine mine is termed an *ophionom*, a helicoid mine is termed a *heliconom*, while a *stigmatonom* is an irregular blotch mine, and so on. The second chapter is devoted to leaf-mines and mines in other parts of plants, while the third chapter discusses "stationary miners" or those insects which pass the whole larval life within the mine and "temporary miners" or those which only form mines during a restricted part of their larval existence. Chapter IV deals with generalities concerning the association of different types of mines with different insects and how to detect them. The seventh chapter is of particular interest in that it discusses larval structure in relation to mining habits. In this connection the excellent work of Trägårdh on the adaptive differences between those Lepidopterous larvae that are sap-feeders, and those that are tissue-feeders, is freely drawn upon. In the succeeding chapter the specialised condition of *Coleophora*, where the body of the larva is enclosed in a case outside the mine, is considered in detail. Chapter X treats of problems concerned with the nutrition of leaf-miners and, in connection with this discussion, there is appended a list of 78 natural orders of plants that are known to be affected by mining insects. The vast majority of the latter pertain to the Diptera and Lepidoptera. Including the Pteridophyta, some 40 natural orders, it seems, are avoided by insects with mining instincts or, at least, no instances of such habit has been recorded from them. Among the remaining chapters those on aquatic mines, and on the parasites, inquilines and symbionts of mining larvae are noteworthy. The book concludes with an extensive bibliography extending over pp. 230–248 and a general index. It is well printed and clearly illustrated, as most German scientific works are, and the price is fairly reasonable considering present-day costs of production.

A. D. IMMS

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